DELETION AND AMBER MUTANTS OF *fla* LOCI IN *ESCHERICHIA COLI* K-12

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ABSTRACT

A rapid screening method for amber *fla* mutants of *E. coli* was devised and many mutants were obtained. In addition, strains with deletions of the *fla* genes in the *his-uvrC* region were isolated from high-temperature survivors of a λcI857 lysogen in which the prophage is located between *his* and *fla.* Utilizing these mutants, eleven *fla* genes (*I—XI*) and one *hag* gene were identified in the *his-uvrc* region, in the following order: *his-supD-I-II-(III, IV)-V-(VI, VII)-VIII-IX-hag-(X, XI)-uvrC*. The *fla* genes *X* and *XI* and *hag* are located at about 42.5 min and the other *fla* genes at about 43.0 min on the *E. coli* genetic map (BACHMANN, LOW and TAYLOR 1976). Mutants of *fla* gene *X* showed a slight sensitivity to *chi* phage, although they lack the flagellar filament.

THE bacterial flagellum is the apparatus which confers motility to the bacterium and allows it to seek a more favorable environment via tactic responses (WEIBULL 1960). Morphological studies have shown that a bacterial flagellum consists of three parts, namely the filament, hook and basal body (DIMMITT and SIMON 1971; DEPAMPHILIS and ADLER 1971a, b, c). They are joined in this order, and the basal body is embedded and anchored in the cell membrane. The filament consists of one kind of polypeptide, the *hag* gene product called flagellin.

Genetic analyses of Salmonella and *Escherichia coli* have revealed that a large number of genes, more than twenty, are involved in the formation of a functional apparatus (IINO 1969, for a review of previous work; YAMAGUCHI *et al.* 1972; VARY and STOCKER 1973; PATTERSON-DELAFIELD *et al.* 1973; SILVERMAN and SIMON 1973a, b and 1974a). The majority of these are termed the *fla* genes, whose defect generally leads to the loss of flagella and which are therefore considered to be concerned with the formation of flagella. However, very little is known about the actual function of the *fla* genes.

In an attempt to clarify the functions of the *fla* genes and to elucidate the process of flagella formation in *E. coli*, we tried to isolate flagellum-defective mutants of the loss-of-product type rather than the loss-of-function type. Obviously, nonsense and deletion mutants fulfill this condition. Such mutants would be useful for identification of the gene products. In addition, complemen-

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tation tests between amber mutants are considered to be free from the complexity due to intracistronic complementation or to interference by mutant products. Polar effects of amber mutations, if present, may throw light on the organized structure of the genes.

We have established a rapid screening method for detecting amber mutants of the *fla* genes in *E. coli*. Furthermore, we have found that deletions in the *his-uvrC* region that include the *fla* genes can be systematically collected by isolating high-temperature survivors of a heat-inducible lambda lysogen in which the prophage is integrated at a secondary attachment site near *his* (SHIMADA, WEISBERG and GOTTESMAN 1972). Using these mutants, we have carried out a genetic analysis of the *fla* genes located in the *his-uvrC* region. This region contains about half of the *fla* genes. The results reported here are consistent with those of others (SILVERMAN and SIMON 1973a, b and 1974a) and offer evidence for the existence of a new *fla* gene which had not been defined previously.

MATERIALS AND METHODS

Bacterial strains: All the bacterial strains used were derivatives of *Escherichia coli* K-12 and are listed in Table 1. Gene designations conform to BACHMAN, LOW and TAYLOR (1976). HKF25, an F-prime factor carrying *his-fla* region, was at first obtained as F'*his* from the cross between Hfr AB311 and a *recA*⁻ female strain KY1340, as described by LOW (1968). HKF25 was found from subsequent crosses to bear some of the *fla* genes but not *uvrC*. KS899 is an Hfr strain which carries a heat-inducible lambda prophage (λcI857) located between *fla* and *his*. HK115 was constructed in the following way. W3623H *fla-100* (*his*⁻, *trp*⁻) was made *fla*+ Su1+ (*supD*⁻)* by cotransduction from CR63. It was then losogenized by φ80*i*λ, which is integrated at the attachment site for φ80 closely linked to *trp*, in order to avoid zygotic induction upon mating in the next step. The *his*⁻, *trp*⁻, Su1+ lysogen thus constructed was then mated with KS899, and *his*+ *trp*+ recombinants were selected on a minimal agar plate containing 250 μg/ml streptomycin at 32°. A few percent of such recombinants turned out to be lysogenic for λcI857, cured of φ80*i*λ, and setill contained the Su1+ marker. HK115 is one such Su1+ *fla*+ strain in which λcI857 is integrated at the same site near *his* as in KS899.

Bacteriophage strains: Chi phage (MEYNELL 1961; SCHADE, ADLER and RIS 1967; gift of DR. T. IINO) was used for selection of *fla*⁻ mutants. φ80pSu3+ (ANDOH and OZEKI 1967) and φ80pSu3[a-23] (SHIMURA et al. 1972) were used for screening for amber *fla* mutants. T4*am*BU33 (GHYSEN and CELIS 1974) was used to test the Su1+ character. P1*vir* (IKEDA and TOMIZAWA 1965) was employed for transduction. Other phages used were, λ, φ80, P2vl, T4D, and BF23.

Media: The following were used. Lambda broth; 1% polypeptone (Daigo), 0.25% NaCl, pH 7. Lambda agar and soft agar; lambda broth supplemented with 1.2% and 0.4% agar (Wako), respectively. P-broth; 1% polypeptone, 0.5% NaCl, pH 7. P-agar and soft agar; P-broth supplemented with 1.2% and 0.35% agar, respectively. PY-broth; 1% polypeptone, 0.5% yeast extract (Difco), 0.5% NaCl, pH 7.2. L-broth; 1% polypeptone, 0.5% yeast extract, 0.1% glucose, 0.5% NaCl, pH 7.2. L-agar and soft agar; L-broth supplemented with 1.2% and 0.35% agar, respectively. M9-glucose medium; 0.58% Na_2PO_4, 0.3% KH_2PO_4, 0.05% NaCl, 0.1% NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 1 μM $FeCl_3$, 2 μg/ml thiamine, 0.4% glucose. Minimal agar; minimal medium of DAVIS and MINGIOLI (1950) supplemented with 0.4% glucose and 1.2% agar. Lambda motility agar; lambda broth supplemented with 0.3% agar. PY-motility agar; PY-broth supplemented with 0.3% agar. PCC-motility agar; 1% polypeptone, 0.5% vitamin-free casamino acids (Difco), 0.2% sodium citrate, 0.28% agar, pH 7. Dilution fluid; 0.1% polypeptone, 0.3% NaCl, pH 7.

* In this paper, Su1+ is employed to designate the phenotype of the active amber suppressor, while *supD*⁻ refers to the genotype and the genetic locus. Thus, both the wild-type (*supD*+) and a deletion mutant of the *supD* locus will appear Su1⁻. Su2+ (*supE*⁻) and Su3+ (*tyrT*⁻) also follow this terminology.

TABLE 1

Bacterial strains

Strain	Pertinent genotypes	Other genotypes	Source and/or references
W3623	*fla*+, *hag*+	*trpA*⁻, *gal*⁻, *str*ʳ	E. LEDERBERG
W3623H	*fla*+, *hag*+, *his*⁻	*trpA*⁻, *gal*⁻, *str*ʳ	*his*⁻ derivative of W3623 isolated after UV-mutagenesis
N17–7	*fla*+, *hag-177**, *uvrC*⁻	*trpA*⁻, *gal*⁻, *str*ʳ	*uvrC*⁻, *hag*⁻ derivative of W3623 (OGAWA, SHIMADA and TOMIZAWA 1968; KONDOH and YANAGIDA 1975)
HK760	*fla*+, *hag*+*, *uvrC*⁻	*trpA*⁻, *gal*⁻, *str*ʳ	*hag*+ transductant of N17–7
HK770	*fla*+, *hag-177*, *uvrC*⁻, *his*⁻	*trpA*⁻, *gal*⁻, *str*ʳ	*his*⁻ derivative of N17–7
W3110†	*fla*+, *hag*+		see BACHMANN (1972)
CR63	*fla*+, *hag*+, *Su1*+ (*supD*⁻)		see BACHMANN (1972)
KS899	*fla*+, *hag*+, (λcI857 near *his*), HfrH.	Δ*gal-uvrB*	K. SHIMADA
HK115	*fla*+, *hag*+, *Su1*+, (λcI857 near *his*)		see MATERIALS AND METHODS
HK900 del	*fla*⁻, HfrH	Δ*gal-uvrB*	*fla* detection strains isolated from KS899
AB311	*fla*+, *hag*+, Hfr (O-*his-trp*--)	*thr*⁻, *leu*⁻, *lac*⁻, *thi*⁻, *str*ʳ	T. YURA, see BACHMANN (1972)
KY1340	*fla*⁻, *hag*+, *recA*⁻, *his*⁻	*met*⁻, *arg*⁻, *trp*⁻*am*, *tyr*⁻*am*	T. YURA, see NAGATA and HORIUCHI (1973)
KHF6	*F′ his*+, *Su1*+	Maintained in KY1340	NAGATA and HORIUCHI (1973)
HKF25‡	F′ *his*+, *fla*+	Maintained in KY1340	see MATERIALS AND METHODS
F′450Su2+	F′ *gal*+, *Su2*+ (*supD*⁻)	Maintained in KY1340	H. INOKUCHI. F450 was made *Su2*+ by E. OHTSUBO

* N17–7 has an additional flagellar defect (probably *che*⁻) which is co-transducible with *hag* but not with *his*. HK760 is a transductant in which both *hag* and the other loci are replaced by wild type alleles.

† In contrast to the reports by VARY and STOCKER (1973) and SILVERMAN and SIMON (1974b), W3110 in our laboratory is *hag*+ and highly motile, as is consistent with its pedigree (BACHMANN 1972).

‡ This F-prime does not cover the *fla* locus of KY1340. Thus, HKF25/KY1340 is *fla*⁻.

Isolation of fla⁻ *mutants*: Most of the mutants were isolated from W3623H as clones resistant to chi phase arising spontaneously or after UV-irradiation (1500 ergs/mm^2). A young culture was infected with chi phase at a multiplicity of 5 and incubated overnight. The chi-resistant cells were plated and colonies were transferred by sterile toothpicks to two lambda motility agar plates, one having been seeded with a mixture of ϕ80pSu3+ and ϕ80pSu3^{a-23} (each 10^9/ml). The nonswarmers on the former plate were assumed to be *fla*⁻ mutants, and, among them, those which did form swarms on the latter plate were assumed to be amber *fla*⁻ mutants. Chi phage-sensitive *fla*⁻ mutants were obtained from a mutagenized culture of W3110. For mutagenesis, cells were grown in lambda broth to a density of 3×10^8/ml, washed in 50 mM Tris-malate buffer (pH 6), and treated with *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (200 μg/ml) in the same buffer at 37° for 30 min. The mutagen-treated cells were washed and grown in lambda broth to saturation.

Cells that failed to migrate from the inoculation site in motility agar were collected after serial enrichments as described by ARMSTRONG, ADLER and DAHL (1967), and examined for sensitivity to chi phage by stabbing in lambda motility agar seeded with chi phage (10^{10}/ml).

Isolation of fla⁻ *deletion mutants*: 0.1 ml of an overnight culture of KS899 in L broth at 30° was plated on L-agar and incubated at 42° until the heat-resistant clones cured of λcI857 grew. Because KS899 is a single lysogen in which λcI857 is integrated at a secondary attachment site, the burst size after incubation at 42° is small, about 10^{-3} per cell, although almost all the cells lyse (see SHIMADA, WEISBERG and GOTTESMAN 1972). Thus, the cured cells escape infection by the phages liberated from the induced cells on the plate. The *fla⁻* deletion mutants were found among the heat-resistant derivatives of KS899. Heat-pulse curing and the measurement of curing frequency were carried out according to SHIMADA, WEISBERG and GOTTESMAN (1972).

Transduction: Recipient cells were cultured overnight in lambda broth, the medium was made 5 mM with $CaCl_2$, and the culture was infected with P1 at a multiplicity of about 0.2. After standing at 37° for 15 min, the mixture was centrifuged and the cells resuspended in dilution fluid were appropriately plated according to the marker selected. When *fla+* was the selected marker, the cell suspension was streaked in lines on a PCC-motility agar plate. In complementation tests between *fla* mutants, "trails" due to abortive transduction were scored after incubation of the motility agar plates at 35° for 24 and 48 hours. *His+* transductants were selected on a minimal agar with appropriate supplements. The distance between two markers was calculated from the cotransduction frequency according to WU (1966) by taking the molecular weight of the DNA's of P1 and *E. coli* as 6×10^7 (IKEDA and TOMIZAWA 1965) and 2.7×10^9 (KLOTZ and ZIMM 1972) respectively.

Mating: Both the male and female strains were grown in PY-broth. Mating occurred under conditions where the female strain was in the stationary phase and the male strain in the logarithmic phase (ca. 2×10^8/ml). When the male strain was an HfrH derivative and *his+* recombinants were selected, equal volumes of the cultures were mixed in a tube to give a female to male ratio of about 10. The mixed culture was left standing at 37° for 70 min, then agitated by a Thermomixer (Thermonics, Tokyo), and plated on minimal agar supplemented with 10 μg/ml tryptophan, 2 μg/ml thiamin and 250 μg/ml streptomycin. When the *fla+* character was the selected marker (e.g., in deletion mapping), 0.01 ml portion of both male and female cultures were mixed directly on a PY-motility agar plate and streaked by the use of sterile paper strips. The plate was kept at 35° for 24 to 48 hours to see if recombinant swarms developed.

Measurement of UV-sensitivity: 0.005 ml portions of a culture were spotted in a linear array in parallel with control cultures (*uvr+* and *uvrC⁻*) on a lambda agar plate. The plate was irradiated under a germicidal lamp (15 W, Toshiba, Tokyo) at an intensity of about 20 erg/$mm^2 \cdot$ sec. Every five to ten seconds, a shield was displaced to cover the spots one by one. The irradiated plate was then kept in the dark at 35° for 20 hours. From the extent of the residual growth compared with the controls, the *uvr* character of each strain was determined.

Growth rate: The mass doubling time of each strain was determined by measuring the increase in absorbance at 550 nm of a logarithmic phase culture using a Beckman DB spectrophotometer.

RESULTS

Isolation of amber fla⁻ *mutants:* Among the *fla⁻* mutants which were isolated as chi phage-resistant non-swarmers in motility agar, those which showed spreading in motility agar inoculated with specialized transducing phages for amber suppressors were classified as amber mutants. We employed Ø80pSu3+ (ANDOH and OZEKI 1968) and its derivative Ø80pSu3^{a-23} (SHIMURA *et al.* 1972) carrying the G82 mutation in the *tyrT* gene. The Su3+ suppressor transfer RNA inserts tyrosine at the position of an amber codon, while the G82 mutation makes it insert glutamine (SMITH and CELIS 1973). Thus, in theory, amber mutants responsive to either one of the two suppressors were recognized. In an experiment

in which 10 independently UV-mutagenized cultures of W3623H were employed, 62 of 500 chi phage-resistant mutants were classified as amber (most of them were *fla⁻* mutants). In addition, suppression of some of the amber mutants turned out to be specific to certain suppressors. This was evident when F-prime factors carrying amber suppressors other than $Su3^+$ were introduced into the cells having amber *fla⁻* mutations (Table 2). The different response to suppressor species is primarily attributed to the difference in the amino acids inserted at the position of amber codon.

Fla⁻ *mutants of the* his-uvrC *region:* We have isolated an F-prime factor, HKF25, carrying *his* and some of the *his*-proximate *fla* genes. HKF25 covers almost all of the *fla* genes of the *his-uvrC* region, but not *uvrC*. Chi phage-resistant mutants were crossed with a *recA⁻* male strain carrying HKF25 on PY-motility agar. About half of the mutants formed swarms either as the result of complementation or of recombination, and hence were classified as belonging to the *his-uvrC* region. Nineteen chi phage-resistant mutants, including fourteen amber mutants, were chosen for further analysis. Amber mutants are indicated by mutant numbers with the prefix *am*. All these mutants lacked flagellar fila-

TABLE 2

Some characteristics of amber fla *mutants*

Mutation	Gene	Sponteous trails†	Swarm production by introduction of:‡				Linkage to *his***
			HKF25	$Su1^+$§	$Su2^+$‖	$Su3^+$¶	
fla-am46	*I*	+	++	—	—	++	
fla-am75	*IV*	—	++	++	++	++	
fla-am69	*VI*	++	++	++	++	+	
fla-am66	*VII*	—	++	±	±	+	
fla-am78	*VII*	++	++	++	++	+	
fla-am87	*VII*	—	++	—	—	++	
fla-am81	*VIII*	+	++	±	±	++	
fla-am4	*VIII*	—	++	+	+	+	
fla-am90	*VIII*	—	++	+	±	+	
fla-am44	*IX*	—	++	++	++	++	
fla-am79	*IX*	—	++	++	++	+	
fla-am76	*X*	—	++	±	++	±	82%
fla-am36	*XI*	—	++	++	++	++	87%
fla-am85	*XI*	—	++	+	±	+	
*fla-am73**		—	—	+	+	+	58%

* This is an example of a *fla* mutation outside of the *his-uvrC* region.

† ++; very long spontaneous trails, comparable in length to those resulted from complementation. +; relatively long spontaneous trails, which are still distinguishable from the trails resulted from complementation. —; no spontaneous trails or, if observed, very short ones.

‡ ++; rapidly spreading swarms. +; slowly spreading compact swarms. ±; bush-like extrusion of cell populations from the position of the inoculum as a result of very weak suppression. —; swarms were not detected.

§ By transfer of KHF6. Serine is inserted at the position of an amber codon.

‖ By transfer of F450Su2+. Glutamine is inserted at the position of an amber codon.

¶ Tests with lysogens of Ø80pSu3+. Tyrosine is inserted at the position of an amber codon.

** Mating with HfrH, *his* being the selected marker. A similar mating experiment with HK770 showed the linkage of *uvrC* to *his* to be 89%.

ments, as revealed by microscopic examination of specimens stained by the method of LEIFSON (1951).

Also included in the analysis was the *hag-177* mutation. This mutation results in the production of straight flagellar filaments (KONDOH and YANAGIDA 1975). The *hag-177* mutation was co-transduced with his^+ from N17–7 to W3623H for further genetic analysis.

A polyhook mutant: We also attempted to isolate mutants having defective flagella, but still sensitive to chi phage. For this purpose, derivatives of W3110 that failed to spread in motility agar were collected and were examined for sensitivity to chi phage as described in MATERIALS AND METHODS. Among a thousand spreading-defective mutants, only ten were highly sensitive to chi phage, and only one of the ten formed swarms upon introduction of HFK25. This one, numbered W3110 *fla-112*, showed a characteristic spinning motion in liquid culture under the phase contrast microscope. Electron micrographs of a specimen negatively stained by phosphotungstate showed that W3110 *fla-112* possesses polyhooks very similar to those reported on *flaE* mutants in *E. coli* (SILVERMAN and SIMON 1972), and *flaR* in Salmonella (PATTERSON-DELAFIELD *et al.* 1973). The length of the polyhook varies from flagellum to flagellum and some of the flagella with polyhooks appear to lack the filament portion entirely, as was the case with the previously reported polyhook mutants. For further genetic analysis, the *fla-112* mutation was cotransduced with the his^+ marker to W3623H, without altering the phenotype.

Complementation between the point mutants: We carried out reciprocal complementation tests between the point mutants by use of P1-mediated abortive transduction. Complementation results in the production of "trails" in motility agar, consisting of almost linearly arranged microcolonies extending from the site of inoculation (see IINO 1969). The majority of the mutants employed were of the amber type. The advantage in the use of amber mutants is the absence of intracistronic complementation, even when only one of the pair of the mutants is of the amber type. Furthermore, polar effects due to amber mutations, if present, would be recognized by weak complementation.

Using a fla^+ strain (W3623) as donor, the length of trails varied considerably from one recipient mutant to another. The length of the trails depended primarily on the distance between the microcolonies. Thus, very short trails often appeared merely as clumps of microncolonies. We encountered such short trails when mutants *fla-am66*, *am81*, and *92* were recipients. Trails produced from the *fla-am66* recipient were especially short. Trail production was not detected when *fla-100* and *hag-177* were the recipients. These mutations may be regarded as dominant over wild-type alleles. With the recipients *fla-52*, *am69*, *am78*, and *112*, the production of abundant and long spontaneous trails interfered with the detection of complementation which might have occurred. For this reason, these mutants were employed only as donors in complementation tests. Spontaneous trails, previously described as "satellite microcolonies" by QUADLING and STOCKER (1957), are attributed to transient leakiness of the mutant phenotype. In PCC-

TABLE 3

Complementation between point mutations

Gene	Gene (Donor) →	I	II	IV	III	V	VI	VII				VIII				IX		hag	X		XI	
Gene	Recipient \ Donor	am 46	95	am 75	52	112	am 69	am 66	am 78	am 87	92	am 81	am 4	am 90	98	am 44	am 79	177	am 76	100	am 36	am 85
I	am46	-	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II	95	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IV	am75	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VII	am66	+	+	+	+	(+)	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
	am87	+	+	+	+	+	+	-	-	-	-	(+)	+	+	+	+	+	+	+	+	+	+
	92	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
VIII	am81	+	+	+	+	+	+	-	+	(+)	+	-	-	-	-	+	+	+	+	+	+	+
	am 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+
	am90	+	+	+	+	+	+	-	(+)	(+)	(+)	-	-	-	-	+	+	+	+	+	+	+
	98	+	+	+	+	+	+	(+)	+	+	(+)	-	-	-	-	+	+	+	+	+	+	+
IX	am44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
	am79	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
X	am76	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+
XI	am36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
	am85	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

+ : Trails were produced, comparable in length to the case with a *fla*+ donor.
(+): Trails were produced, but shorter than in the case of a *fla*+ donor.
— : Trails were not detected.
* The following could not be employed as recipients: *fla-52, 112, am69*, and *am78* because of long and numerous spontaneous trails; *fla-100* and *hag-177* because trails were not produced even with a *fla*+ donor.

motility agar spontaneous trails were not so different morphologically from trails due to transductants.

The degree of complementation between mutants was scored as the relative length of trails produced from the same recipient compared with the case in which *fla*+ was the donor. The results are summarized in Table 3. Pairs with negative complementation are grouped into genes. In the course of grouping the mutants, positive complementation between amber mutants, however weak, was taken as evidence for the two mutants being in different genes. Thus we classified the recipient mutants into eight genes. In addition, *fla-52, 112,* and *am69,* employed only as donors and assigned to genes different from the above eight, were separated into three groups, since *fla-am69* complemented with deletion mutant *del12*, whereas *fla-112* did not, and also since *fla-112* but not *fla-52* did complement with *del15* (see below). Thus, eleven *fla* genes were defined within *his-uvrC* region. We tentatively designate these *fla* genes by Roman numerals,

flaI to *flaXI*, in order to avoid confusion with those defined and alphabetically designated by SILVERMAN and SIMON (1973a, b, 1974).

The *hag* mutant *hag-177*, employed only as a donor, complemented with all *fla* mutants testable as recipients except for *fla-am76* (*flaX*). This result suggested that *hag* might be identical to *flaX*. However, complementation tests with *del55* as recipient differentiate *hag-177* and the mutants in *flaX* (see below).

The complementation between the mutants of *flaVII* and *flaVIII* was often weak or appeared negative, especially in the cases in which the mutant *fla-am66* was the recipient. This fact is reflected in Table 3 as apparently nonreciprocal complementation. The two *fla* genes *VII* and *VIII* were found to be located near each other (see below). The weak complementation between the amber mutants in these genes may be ascribed to the polar effect of amber mutations, and thus suggests that these two *fla* genes belong to the same transcriptional unit.

Sensitivity of the mutants to chi *phage:* We reexamined the *chi*-sensitivity of the mutants in detail. The strains having the *hag-177* mutation were indistinguishable from the fla^+ hag^+ strain (W3623) in plating efficiency and the plaque morphology of chi phage (clear plaques). The strains having the *fla-112* mutation (polyhook) were slightly resistant, but still allowed chi phage to form plaques. The efficiency of plating was reduced to about 0.2 relative to the fla^+ strain and the plaques were turbid with indistinct contours. Among the rest of the mutants, which lacked filaments, only the strains with *fla-am76* or *fla-100* mutations showed a slight sensitivity to chi phage as revealed by spot tests; there was no clearing of the spotted area but partial lysis with a halo was observed.

Since both of these mutations are assigned to the same gene, *flaXII*, this type of sensitivity to chi phage might be taken as the characteristic phenotype of the mutants in this gene.

Isolation of deletions of the fla *genes of the* his-uvrC *region:* It has been known that high-temperature-resistant strains derived from heat-inducible λ lysogens carry deletions of the prophage and that the deletions very often extend into the adjacent bacterial genes (SHAPIRO and ADHYA 1969; SHIMADA, WEISBERG and GOTTESMAN 1972; SCHLEIF 1972). KS899, a derivative of HfrH, which we owe to K. SHIMADA, has a deletion of the primary attachment site for λ, and heat-inducible λcI857 (SUSSMAN and JACOB 1962) is integrated at one of the secondary attachment sites located on the *uvrC* side of *his*. The high-temperature survivors therefrom may bear deletions extending to *fla* genes.

A 0.1 ml portion from an overnight culture of KS899 in L-broth at 30° was plated on L-agar, and the plate was incubated at 42° for 24 to 36 hours until the heat-resistant colonies grew. Incubation of the culture at 42° for a brief period (heat pulse) prior to the selection at 42° enhanced the frequency of the surviving cells by a factor of ten to fifty. By testing the heat-resistant colonies on PY-motility agar, fla^- mutants were found, although the frequency of appearance of fla^- varied considerably from one experiment to another (Table 4). The fla^- character thus obtained could be transferred to W3623H by mating. Among the his^+ recombinants selected, 80 to 90% were at the same time fla^-, the linkage being comparable to fla^- point mutations covered by F-prime factor HKF 25 and

TABLE 4

Occurrence of fla⁻ *and* his⁻ *cells among high-temperature survivors of KS899*

Experiment number	Curing of lambda	Curing frequency	Frequency among the high temperature survivors		
			fla⁻	*his*⁻	*fla*⁻, *his*⁻
1	spontaneous	3×10^{-7}	3% (1/31)	6% (2/31)	0
2	spontaneous	ND*	11% (11/100)	ND	
3	heat-pulse	5×10^{-6}	49% (47/96)	3% (3/96)	0
4	heat-pulse	2×10^{-6}	0% (0/100)	2% (2/100)	0
5	heat-pulse	1×10^{-5}	17% (15/90)	0% (0/90)	0

* Not determined.

also to *uvrC* (Table 2). This fact strongly suggests that the *fla*⁻ character of the KS899 derivatives is due to deletions extending from λ prophage to the *fla* genes. These *fla*⁻ mutants were in fact found to be deletion mutants (see below); they were designated the HK900 series and numbered serially with the prefix *del.* When HKF25 was transferred to the strains having various *fla* deletions (genetic background being W3623), all of them formed swarms, indicating that the deletions are covered by this F-prime factor. In addition, these deletion mutants show UV-sensitivity comparable to W3623. The deletions, therefore, do not extend to the *uvrC* gene.

Also found among the high-temperature survivors were some *his*⁻ auxotrophs (Table 4). In no case were *his*⁻ *fla*⁻ mutants found. Thus it was concluded that no *fla* gene is present between the *his* genes and the secondary attachment site for λ in KS899.

In order to know the extent of the deletions of the HK900 series in the *his* direction, we attempted to position the prophage site in KS899 relative to the *supD* (Su1) locus. From the principle of the selection procedure for the deletion strains, the deletions should include the prophage site. First, P1 transduction was carried out employing the *fla*⁺ Su1⁺ strain (CR63) as donor and several deletion mutants as recipients. All the *fla*⁺ recombinants became at the same time Su1⁺. Second, strain HK115 with the following characteristics was con-

TABLE 5

*Characteristics of high-temperature survivors of HK115**

Character		Number
his⁻		0
his⁺ *fla*⁻	Su1⁻	9
	Su1⁺	0
his⁺ *fla*⁺	Su1⁻	3
	Su1⁺	31
Total		43

* Heat-pulsed prior to selection.

structured; *fla*$^+$, Su1$^+$ and lysogenic for λcI857 at the same location as in KS899. From HK115, heat-resistant derivatives were selected and their characteristics determined. As shown in Table 5, all the *fla*$^-$ derivatives were Su1$^-$ without exception. In contrast, both Su1$^+$ and Su1$^-$ strains were found among the *fla*$^+$ derivatives. Thus only the order, *his-λ-supD-fla*, is compatible with the results.

None of the deletions of the HK900 series showed an additional auxotrophy. Nevertheless, some deletions may affect the growth of the cells and bacteriophages. The growth rate of the deletion mutants in the logarithmic phase was compared with otherwise isogenic nondeletion strains. Growth in L-broth was indistinguishable for the strains with and without deletions. However, a remarkable difference was observed in a minimal medium (M9-glucose). The strains carrying deletions grew slowly, with a doubling time almost twice as long as that of the nondeletion strains (Table 6). Since the strains with *del4* and *del10* (long and short deletions, see below) have the same growth rate, the slow growth in minimal medium may be ascribed to the loss of the region between λ and *fla*, but not to the *fla* region itself. On the other hand, we could not find any effect of the deletions on the multiplication of phages. The following bacteriophages were indistinguishable on strains W3623 and W3623 *del4* as far as plating efficiency and plaque morphology were concerned: λ, Ø80 (on lambda agar), P1*vir*, P2vl, BF23 (on L-agar supplemented with 5mM $CaCl_2$), and T4D (on P-agar).

Deletion mapping by mating: The Hfr's having *fla*-deletions (HK900 series) were mated with F$^-$ strains having *fla* point mutations to see if swarms due to *fla*$^+$ recombinants develop. The results are summarized in Table 7. Most of the Hfr strains of the HK900 series failed to produce *fla*$^+$ recombinants with more than one *fla*$^-$ point mutant. This indicated that the *fla* character of the HK900 series resulted from deletions. Assuming that all the deletions start from the *his*-proximate side of the *fla* genes, the point mutation sites could be arranged linearly on the basis of the above results. The order of the mutational sites is consistent with the complementation groups defined in the foregoing section.

The *his*-distal end points of the deletions divided the *fla* region into eight segments. In addition, the segment between ED92 and ED52 (where ED refers to the *e*nd point of *d*eletion in the *fla* genes) was subdivided into two parts according

TABLE 6

Growth of fla*-deletion strains*

Strain	Doubling time in: L-broth	Doubling time in: M9-glucose*
HfrH Δ*gal-uvrB*	30 min	90 min
HfrH Δ*gal-uvrB del10* (HK900 *del10*)	30	165
W3623	25	85
W3623 *del10*	25	155
W3623 *del4*	25	155

* Supplemented with 0.5 μg/ml biotin (upper lines) or 10 μg/ml tryptophan (lower lines).

TABLE 7

Deletion mapping by mating

Hfr \ F⁻ Gene	I	II	III	IV	V	VI	VII				VIII				IX		hag	X		XI		*
	am 46	95	52	am 75	112	am 69	am 66	am 78	am 87	92	am 81	am 4	am 90	98	am 44	am 79	177	am 76	100	am 36	am 85	am 73
del54	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
del10	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
del15	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
del12	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
del92	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
del52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
del55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
del4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

+: *fla*+ recombinants were produced; —: *fla*+ recombinants were not detected.
* *am73* maps outside the *his-uvrC* region (see Table 2).

to the recombinant frequency. In matings with HK900 *del92*, *fla-am44* and *fla-am79* (gene *IX*) produced more than 50 recombinant swarms in 24 hours in contrast to the other four-point mutants of the same segment (i.e., *fla-am81*, *am4*, *am90*, *98* (gene *VIII*)) which produced only 10 to 15 swarms. Similar results were obtained in triplicate experiments, indicating that *flaIX* is more distant from ED92 than *flaVIII*.

We did not detect swarms above the revertant level in the cross between *hag-177* and *del55*. However, it is difficult to imagine that the *hag* gene is deleted in *del55*, since *hag-177* complements *del55*, although weakly (see below). It seems that ED55 is very close to the *hag-177* mutation site.

Thus the following order of the mutation sites and the genes was concluded:

his—*flaI*(*am46*)—ED54—*flaII*(*95*)—ED10—{*flaIII*(*52*), *flaIV*(*am75*)}—ED15—*flaV*(*112*)—ED12—{*flaVI*(*am69*), *flaVII*(*am66*, *am87*, *92*)}—ED92—*flaVIII*(*am81*, *am4*, *am90*, *98*)—*flaIX*(*am44*, *am79*)—ED52—ED55—*hag-177*—{*flaX*(*am76*, *100*), *flaXI*(*am36*, *am85*)}—ED4—*uvrC*.

The order of gene pairs in braces is not known. It is to be noted that the genes *VII* and *VIII*, the mutants of which often complemented each other weakly (see Table 3), are localized within a small region. This fact is compatible with the suggestion that the two genes belong to a single transcriptional unit.

Complementation between point and deletion mutants: We carried out complementation tests between point and the deletion mutants by use of P1-mediated transduction. This test was another method of deletion mapping, and was expected to reveal some aspects of the genetic structure which had not been evident from the mating experiments. At first, a *fla*+ strain was employed as donor and the deletion mutants as recipients, not only because of absence of

TABLE 8

P1 transduction of the Fla+ *character to* fla *deletion strains with a* fla+ *donor*

Recipient	Number of transductants per 2×10^8 infective centers	
	Abortive transductants	Complete transductants
del54	170	3
del10	183	4
del15	130	2
del92	114	3
del52	120	2
del55	113	2
del4	111	1

revertants but also because of absence of spontaneous trails. The frequency of appearance of transductants with various deletion mutants is presented in Table 8. Almost all the transductants were abortive ones and the ratio of abortive to complete transductants was as high as about 50:1 in contrast to the value of 5:1 which is usually observed when recipients are *fla* point mutants. The low frequency of complete transductants even in the case of the shortest *fla* deletion *del54* may indicate that the deletion extends from the *fla* genes a relatively long distance to *his* side.

The results of transductions employing point mutants as donors are summarized in Table 9. The pattern of complementation was similar to that of recombinant formation in Table 7, but not the same.

Among the pairs which failed to form appreciable numbers of recombinants upon mating, we found that *hag-177* and *del55* complement each other. As was

TABLE 9

Complementation between point and deletion mutations

Gene	I	II	III	IV	V	VI	VII				VIII				IX		hag	X		XI	
Donor / Recipient	am 46	95	52	am 75	112	am 69	am 66	am 78	am 87	92	am 81	am 4	am 90	98	am 44	am 79	177	am 76	100	am 36	am 85
del54	-	-	+	+																	
del10	-	-	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+
del15	-	-	-	-	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+
del12		-	-	-	-	(+)	(+)	(+)	(+)	+	+	+	+	+	+	+	+	+	+		+
del92					-	-	-	-	-	-	(+)	(+)	(+)	+	+	+	+				
del52															-	-	+	+	+	+	+
del55															-	-	*	+	+	+	+
del4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Trails were produced at about one tenth the frequency of the cases indicated by the + sign. The other signs accord with those of Table 3. The solid line indicates the result of deletion mapping by mating.

mentioned in the foregoing section, this suggests that the *hag-177* site and the end point of *del55* are very close. In the case of this pair, however, the frequency of trail production was as low as one-tenth the usual in transductional complementation. Such an effect might be manifested if the expression of the *hag* gene is reduced to a low level in the *del55* mutant. On the other hand, when mutants of the *flaX* gene, *fla-am76* and *100*, were the donors, trails were produced with the usual frequency from *del55* as recipient. These facts strongly suggest that *hag* and *flaX* are distinct genes.

In the case of the *fla-95* and *del54* pairs, abortive transductants were not detected, in contrast with the recombinants formed upon mating. This fact indicates that the deletion terminates within the same gene (if transcription is from the *uvrC* to the *his* side) or within the same transcriptional unit (if the transcription is in the reverse direction) as the mutational site of *fla-95*.

In some instances where amber mutants were donors the trails were relatively short, as indicated by (+) in Table 9. The phenomenon of short trail production might be brought about by a polar effect of the amber mutations. With this assumption, the results in Table 9 can be interpreted as follows. Amber mutations in gene *VIII* exercise their polar effect on the expression of the genes which are lacking in *del92* but are present in *del12*, that is, *flaVI* and *flaVII*. Similarly, the polar effect of amber mutations in *fla* genes *VI* and *VII* affect the expression of the gene lacking in *del12* but present in *del15*, that is, *flaV*. Putting these interpretations together, it is concluded that the four *fla* genes, *V*, *VI*, *VII* and *VIII*, constitute a polycistronic unit transcribed leftward. This interpretation is compatible with the results of complementations between point mutants (Table 3). Many combinations of the amber mutants of *flaVII* and *flaVIII*, and, in addition, *fla-112* (gene *V*) and *fla-am66* (gene *VII*) complemented only weakly.

Cotransduction of fla *and* hag *with other markers:* In order to determine the position of the *fla* and *hag* genes on the genetic map, the cotransduction frequencies of *fla* and *hag* mutational sites with other markers were measured. For such measurements, *uvrC*, *supD* and *his* were available. The results are summarized in Tables 10, 11 and 12. From the cotransduction frequencies with *uvrC*,

TABLE 10

Cotransduction of uvrC *with* fla *or* hag

Recipient	No. of fla^+ or hag^+ recombinants	No. of fla^+ (hag^+) and $uvrC^-$ recombinants	Cotransduction frequency
W3623H *fla-am85*	52	31	60%
W3623H *fla-am76*	30	20	67
W3623 *hag-177*	18	10	56
W3623H *fla-am79*	80	18	23
W3623H *fla-am4*	47	11	23
W3623H *fla-am87*	55	14	25
W3623 *fla-112*	32	8	25
W3623H *fla-95*	50	12	24

Donor: HK760; fla^+, hag^+, $uvrC^-$.

TABLE 11

Cotransduction of supD *with* fla

Recipient	No. of *fla*+ recombinants	No. of *fla*+ and Su1+ recombinants	Cotransduction frequency
W3623H *fla-am76*	105	5	4.8%
W3623H *fla-100*	63	6	9.5
W3623H *fla-98*	82	11	13
W3623H *fla-92*	82	8	9.8
W3623H *fla-am87*	95	12	13
W3623 *fla-112*	23	4	17
W3623H *fla-52*	74	15	20
W3623H *fla-95*	80	13	16

Donor: CR63; *fla*+, Su1+ (*supD*).

TABLE 12

Cotransduction of fla, hag *and* supD *with* his

Donor	Recipient	No. of *his*+ recombinants	No. of recombinants with unselected donor marker	Cotransduction frequency
N17-7 (*his*+, *hag-177*)	W3623H (*his*−)	300	6	2.0%
W3110 *fla-112*	W3623H	100	2	2.0
W3623	W3623H *fla-am36*	267	6	2.2
W3623	W3623H *fla-am44*	441	10	2.3
W3623	W3623H *fla-52*	408	23	3.8
CR63 (*supD*−)	W3623H	101	9	9.0

the *fla* and *hag* loci were divided into two map positions. All the mutant loci deleted in mutant *del52* were concentrated in a site near 43.0 min, and the rest were at about 42.5 min on the *E. coli* map, assuming *uvrC* is at 42.3 min (BACHMANN, LOW and TAYLOR 1976). The *fla* and *hag* genes located between *his* and *uvrC* thus appear to comprise two clusters 0.5 min apart. Cotransduction frequencies with *supD* and *his* markers were also consistent with these map positions. The *supD* locus would be slightly closer to *fla-95* than to *his*.

DISCUSSION

We have devised a rapid screening method for amber *fla* mutants by the use of specialized transducing phages carrying amber suppressor genes. In addition, a male strain in which heat-inducible λ prophage is located at one of the secondary attachment sites near *his* provided a very useful system for the isolation of *fla*-deletion strains. Mutants with various deletions spanning the *fla* region between *his* and *uvrC* were found among the survivors of an exposure to high temperature. By extensive use of strains with amber and deletion mutations, we have carried out a genetic analysis of the *fla* genes in the *his-uvrC* region, identifying eleven *fla* genes and a *hag* gene. The genetic structure of this region deduced from our analysis is summarized in Figure 1. SILVERMAN and SIMON

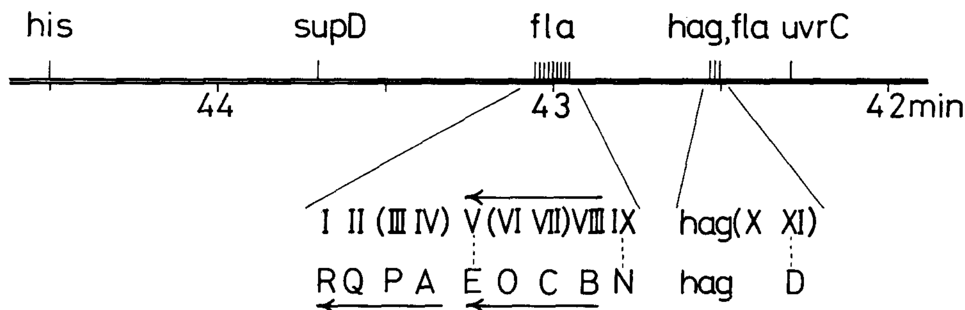

FIGURE 1.—Summary of the genetic structure of the *his-uvrC* region of the *E. coli* map. The Roman numerals indicate the *fla* genes defined in this work, the arrows the polycistronic transcriptional units and the direction of their transcription. Capital letters are the *fla* genes defined by SILVERMAN and SIMON (1974a), and the dotted lines indicate the possible correspondences of the genes.

(1973a, b and 1974a) have analyzed the *fla* genes independently. Their results are compared with ours in the same figure. We designate the *fla* genes by Roman numerals, since the correspondence with those defined by SILVERMAN and SIMON has not been firmly established. We discuss below in detail the *fla* genes of the *his-uvrC* region as revealed by our analysis and also from comparison with the data of others. For convenience we divide the region into five parts according to deletion end points (ED).

The region between uvrC *and ED4:* No *fla* mutations in our collection have been mapped in this region.

The region between ED4 and ED52: We have identified two *fla* genes, *X* and *XI*, and a *hag* gene in this region. SILVERMAN and SIMON (1973b, 1974a), on the other hand, found only *flaD* and *hag* genes in the corresponding region. Since the mutants in *flaX* do not complement the *hag* mutant, the discrepancy seems to have originated from whether or not *flaX* and *hag* are distinguished. The major argument for the distinction between *flaX* and *hag* stands on the fact that the *hag* mutant complements with *del55* only at low efficiency, in contrast with the complementation between *flaX* mutants and *del55* at the normal level. Recently we extended the complementation test with newly isolated mutants of *hag* and *flaX*, and again observed such a difference (KONDOH, unpublished). The expression of the *hag* gene, but not the *flaX* gene, is reduced in the *del55* mutant. However, it should be remembered that the *hag* mutants employed are abnormal only in the gross morphology of flagellar filaments, and normal with respect to the processes of synthesis and assembly of flagellin. It is possible that *hag* mutants defective in the synthesis or assembly of flagellin could behave differently from morphological mutants in complementation tests.

Another line of argument of a distinct *flaX* gene is drawn from comparison of the *E. coli* and Salmonella *fla* genes. In Salmonella, the *flaL* gene maps closely to the right of H1, corresponding to *hag* in *E. coli* (YAMAGUCHI *et al.* 1972). TSUI (1974) tentatively assigned *flaK* (Salmonella), instead of *flaL*, to be the gene corresponding to *flaD* in *E. coli*. No *E. coli fla* gene corresponding to *flaL*

has been described. It should be recalled that *flaX* mutants are characterized by slight sensitivity to chi phage. Now it is known that mutants of *flaL* share the same phenotype with those of *flaX* (IINO, personal communication). Thus it is likely that *flaX* corresponds to *flaL* in Salmonella, and hence that *flaXI* corresponds to *flaD*.

Chi phage injects its DNA at the base of the flagellum. Therefore, the basal parts of flagellum should have taken shape in *flaX* mutants. It is possible that the *flaX* gene has regulatory functions for flagellin synthesis. This might account for the absence of complementation between *hag* and *flaX* mutants.

Recently we isolated specialized transducing phages carrying the genes of this region (KONDOH, unpublished). Analysis by use of these phages may settle the problem.

The region between ED52 and ED15: This region, to which five *fla* genes belong, is subdivided by ED92 and ED12. The gene at the right end of this cluster, *flaIX*, seems to correspond to *flaN* as defined by SILVERMAN and SIMON (1973a, 1974a) in light of its map position. The left end *fla* gene, *V*, is represented by the *fla-112* mutation. The polyhook phenotype and the map position of this mutation suggest that *flaV* corresponds to *flaE* in *E. coli* (SILVERMAN and SIMON 1972, 1974a) and to *flaR* in Salmonella (PATTERSON-DELAFIELD *et al.* 1973). Our recent data have shown that *flaVI* is to the left of *flaVII*. Thus, the complete order of the *fla* genes is, *V–VI–VII–VIII–IX*. SILVERMAN and SIMON (1973b and 1974a) defined the same number of *fla* genes, *E, O, C, B* and *N* in the corresponding region.

Amber mutants of the *fla* genes *VII* and *VIII* complemented each other and deletion mutants *del12* and *del92* only weakly, probably as a result of polar effects. These genes seem to belong to the same polycistronic transcriptional unit. In addition, weak complementation between *fla-am69* and *del12* and also between *fla-am66* and *fla-112* suggest that *fla* genes *V* and *VI* are part of the above unit as well. The transcription of this unit would be from right to left. SILVERMAN and SIMON (1973b and 1974a) found a leftward transcriptional unit starting from *flaB* and terminating after *flaE*. The size and direction of this transcriptional unit are compatible with our results. In Salmonella only three *fla* cistrons are defined between *flaR* and H1 (YAMAGUCHI *et al.* 1972; PATTERSON-DELAFIELD *et al.* 1973).

The region between ED15 and the left side of the fla *cluster:* Four *fla* genes from *I* to *IV* are defined in this region. Since our recent data have shown that *flaIII* is to the left of *flaIV*, the gene order accords with the numerical order. SILVERMAN and SIMON (1973b and 1974a) have defined the same number of *fla* genes, *R, Q, P,* and *A,* which comprise a leftward transcriptional unit. In Salmonella, however, five *fla* genes have been found in the region to the left of *flaR* (YAMAGUCHI *et al.* 1972; PATTERSON-DELAFIELD *et al.* 1973).

The region between fla *and the* λ *attachment site of KS899:* It was established that the *supD* locus resides in this region. The DNA cytosine methylase (*dcm*) gene mapping between *fla* and *supD* (MARINUS 1973) should be lost in the deletion mutants used in this work. Nonlethality and a sensitivity to UV com-

parable to that of wild type in these deletion mutants confirms the conclusion drawn from the *dcm* point mutants (MARINUS and MORRIS 1973) and from the hybrid strain with *E. coli* B (MAMELAK and BOYER 1970). Two missense suppressor loci, *supH* (EGGERTSSON and ADELBERG 1965) and *glyW* (FLECK and CARBON 1975), seem to be deleted as well. Some other genes for tRNA's may be also present in this region. It should be mentioned that various deletions of this region, which can be isolated in a similar way to *fla*-deletions, can provide a useful genetic system for the analysis of tRNA genes in this region. In *Salmonella typhimurium, supD* showed a cotransduction frequency of about 40% with the nearest *fla* marker with the P22 system (FANKHAUSER and HARTMAN 1971), which is higher than in the case of the *E. coli*-P1 transduction system (about 20%), in spite of the fact that the DNA content of a P22 particle is only about 40% that of P1. Thus, the *supD-fla* segment of the *S. typhimurium* genome might be shorter than that of the *E. coli* K-12 genome.

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