DEFINITION OF ADDITIONAL FLAGELLAR GENES IN *ESCHERICHIA COLI* K12

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ABSTRACT

Twenty-nine flagellar genes in *Escherichia coli* K12 have previously been assigned to three regions of the genome. Flagellar region I is located between *pyrC* and *ptsG*, region II between *aroD* and *uvrC*, and region III between *uvrC* and *his*. In this study, flagellar mutants in *Escherichia coli* K12 were obtained by selection for resistance to the flagellotropic phage, χ. They were analyzed in complementation tests using P1 phage-mediated transduction. In addition to the *fla* genes already described, eight more flagellar genes were identified. This analysis defined six more *fla* genes in region I (*flaU*, etc.), one more in region II (*flbB*) and one more in region III (*flbC*). Region I was shown to include at least 12 *fla* cistrons. Complementation analysis with polar Mu phage-induced Fla⁻ mutants and with λ*fla* phage defined four transcriptional units in region I. These were: *flaU*, *flbA-flaW-flaV-flaK-flaX-flaL-flaY-flaM*, *flaZ* and *flaS- flaT*, with transcription proceeding from left to right. The *flbB* gene was found to be part of an operon: *flbB-flaI* in region II. In region III, a previously unidentified gene *flbC* was located between *hag* and *flaN*.

MOTILITY and chemotaxis in bacteria result from flagellar organelle function. Genetic analyses of flagellar mutants have defined a large number of genes involved in the formation of a functional flagellar apparatus (IINO 1977; SILVERMAN and SIMON 1977b). Extensive studies have been performed in Salmonella and *Escherichia coli* K12. More than 25 flagellar genes in Salmonella (IINO 1977; SUZUKI *et al.* 1978) and 19 genes in *E. coli* (SILVERMAN and SIMON 1977b; KOMEDA, SILVERMAN and SIMON 1977) have been identified. In *E. coli*, *fla* genes have been assigned to three regions of the genome (SILVERMAN and SIMON 1973a). The region I *fla* genes were located between *pyrC* and *ptsG*; those of region II between *aroD* and *uvrC*, and those of region III between *uvrC* and *his*.

Using intergeneric crosses, functional homology has been shown with respect to the *fla* genes of *Salmonella typhimurium* and *E. coli* (KUTSUKAKE *et al.*, unpublished data). These experiments also suggested the existence of additional *fla* genes in *E. coli*. Therefore, we have extensively analyzed flagellar mutants in *E. coli*. These mutants include spontaneous, Mu phage-induced and some transposon-induced flagellar mutants. Complementation analysis employed P1 phage-

mediated transduction. We could identify six additional genes in region I, one more in region II and another one in region III. This analysis revealed four additional flagellar gene operons.

MATERIALS AND METHODS

Organisms: The *E. coli* K12 strains used are shown in Table 1. Flagellar mutants used for genetic analysis are described in the text or figures. The P1 phage used for transduction was a P1kc derivative that could grow on *galU* mutants (KOMEDA, ICHO and IINO 1977). λ*fla* phages used for mapping are described in Table 2.

Media: Tryptone broth contained (per liter of distilled water): tryptone (Difco), 10 g; NaCl, 5 g. L broth contained (per liter of distilled water): tryptone, 10 g; NaCl, 5 g; yeast extract (Difco), 5 g; and thymine, 0.1 g. L agar plates were prepared by adding 1.2% agar (Shoei) to L broth. Motility agar plates were prepared by adding 0.25% agar to tryptone broth with supplements of 0.1 g of thymine and uracil per liter. Minimal medium contained (per liter of distilled water): K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; sodium citrate·$2H_2O$, 0.47 g; glucose, 4 g; and thiamine, 1 mg. Amino acids and bases, if required, were added to a final concentration of 1 mM. Minimal agar plates were prepared by adding 1.2% agar to the minimal medium.

Isolation of flagellar mutants: In addition to the Fla⁻ mutants already described (SILVERMAN and SIMON 1973a; KOMEDA, SILVERMAN and SIMON 1977), three kinds of flagellar mutants were isolated. These were spontaneous, Mu phage-induced and transposon-induced flagellar mutants. Spontaneous and Mu phage-induced Fla⁻ mutants were isolated as described previously (KOMEDA, SILVERMAN and SIMON 1977). The Mu phage used was Mucts62 (HOWE 1973) or Mud1(Ap^r, *lac*) (a generous gift of M. J. CASADABAN). Precise characterization of the mutants

TABLE 1

Bacterial strains used

Strain	Relevant genotypes	References
EJ500	F⁻ *cfs*	ICHO and IINO (1978)
CSH26	F⁻ *ara*, Δ(*lac*, *pro*), *thi*	J. H. MILLER
MC4100	F⁻ *araD139*, Δ(*lac*)U169, *strA*, *thi*, *fla*	CASADABAN (1976)
YK202(=159λ)	F⁻ *galK2 uvrA157 rpsL200* (λ*ind*⁻)	SILVERMAN *et al.* (1976)
YK405	F⁻ *araD139*, Δ(*lac*)U169, *strA*, *thi*, *pyrC46*, *nalA*, *thyA*	This study
YK410	F⁻ *araD139*, Δ(*lac*)U169, *strA*, *thi*, *pyrC46*, *nalA*, *thyA*, *his*	This study
YK2801 series	F⁻ *fla*(am) of YK202	This study
YK3201~3216	F⁻ *fla*::(Mucts) of CSH26	This study
YK3217~	F⁻ *fla*::(Mucts) of YK405	This study
YK3301 series	F⁻ *fla*::(Mucts) of YK410	This study
YK3401 series	F⁻ *fla*::Mud(Ap^r, *lac*) of YK405, (*lac*+ by operon fusion of *fla* to *lac*)	This study
YK4001 series	F⁻ *fla* of CSH26	This study
YK4101 series	F⁻ *fla* of YK410	This study
YK4301 series	F⁻ *fla*::Mud(Ap^r, *lac*) of YK410, (*lac*+ by operon fusion of *fla* to *lac*)	This study
YK4401 series	F⁻ *fla* of YK410	This study
YK4501 series	F⁻ *fla*::Tn*5* or *fla*::Tn*10* of YK410	This study
JA200(pLC series)	F+ *trpE5*, *recA*, *thr*, *leu*, *thy*, *lacY*	CLARKE and CARBON (1976)

TABLE 2

λ fla *phages*

Strain	Relevant *E. coli* genes carried	Source and/or references
λ*fla691*	*flaV*, *flaK*, *flaL*, *flaM*, *flaS*, *flaT*	KOMEDA, SILVERMAN and SIMON (1977)
λyk3	*flaG*, *flaH*	Y. KOMEDA (unpublished)
λ*fla22*(λ*fla2*)	*flaI*, *motA*, *motB*, part of *cheA*	SILVERMAN *et al.* (1977)
λ*fla52*, λ*fla57*	*motA*, *motB*, *cheA*, *cheW*, *cheM*, *cheX*	SILVERMAN and SIMON (1977a)
λd*fla#7–11*	*hag*, *flaD*	KOMEDA, SHIMADA and IINO (1977)
λ*fla1*Δ4	*hag*	SILVERMAN *et al.* (1977)
λ*fla#36*	*flaN*, *flaB*, *flaC*, *flaO*, *flaE*, *flaA*, *flaP*, *flaQ*, *flaR*	KOMEDA, SHIMADA and IINO (1977)
λ*fla18*	*cheA*, *motB*, *motA*, *flaI*, *hag*	SILVERMAN and SIMON (1977a)
λ*fla23*, λ*fla42*	*cheY*, *cheB*, *cheX*, *cheM*, *cheW*, *cheA*, *hag*	SILVERMAN and SIMON (1977a)

induced with Mud1(Ap^r, *lac*) will be described in another report. Mu phage-induced Fla$^-$ mutants were saved if transduction to Fla$^+$ resulted in simultaneous loss of the Mu phage. Transposon-induced mutagenesis was performed as described previously (HARAYAMA, PALVA and HAZELBAUER 1979; KLECKNER *et al.* 1978). Out of 4,800 Tn*5*(Km^r)-induced and 1,300 Tn*10*(Tc^r)-induced mutants from strain EJ500, 10 and 4 flagellar mutants were isolated, respectively. They were used as donor for P1-mediated transduction into strain YK410. Drug-resistant transductants were examined for *fla* mutations, and the Fla$^-$ mutants were saved.

The strain designation for Fla$^-$ mutants is also shown in Table 1 along with the parental strains.

Complementation analysis: Complementation analysis was performed by using P1 phage-mediated transduction. An overnight culture of each recipient Fla$^-$ mutant was adjusted to 5 mM $CaCl_2$ and mixed with P1 phage grown on the donor Fla$^-$ mutant at a multiplicity of infection of 0.1. The mixture was incubated at 37° for 1 hr. This transduction mixture was brushed on motility agar plates with a pipette. The plates were examined for trail formation after 20 hr of cultivation (LEDERBERG 1956; STOCKER 1956). The complementation behavior of Fla$^-$ mutants derived from strain YK405 or YK410 could be analyzed by the cross-streak method as described for λ phage-mediated transduction (SILVERMAN, MATSUMURA and SIMON 1976) after overnight incubation at 37°.

RESULTS

Mutations in flagellar mutants were located on the genome of *E. coli* by crosses using the λ-*fla* phages shown in Table 2. Subsequent complementation analysis employed P1 phage-mediated transduction.

Complementation analysis of region I flagellar mutants: Previous genetic analysis (KOMEDA, SILVERMAN and SIMON 1977) had identified six cistrons in region I (see Figure 4) comprising one transcriptional unit (or operon according to the definition of N. FRANKLIN (1978)). KUTSUKAKE *et al.* (unpublished results) obtained evidence suggesting that the *flaK* gene could be divided into

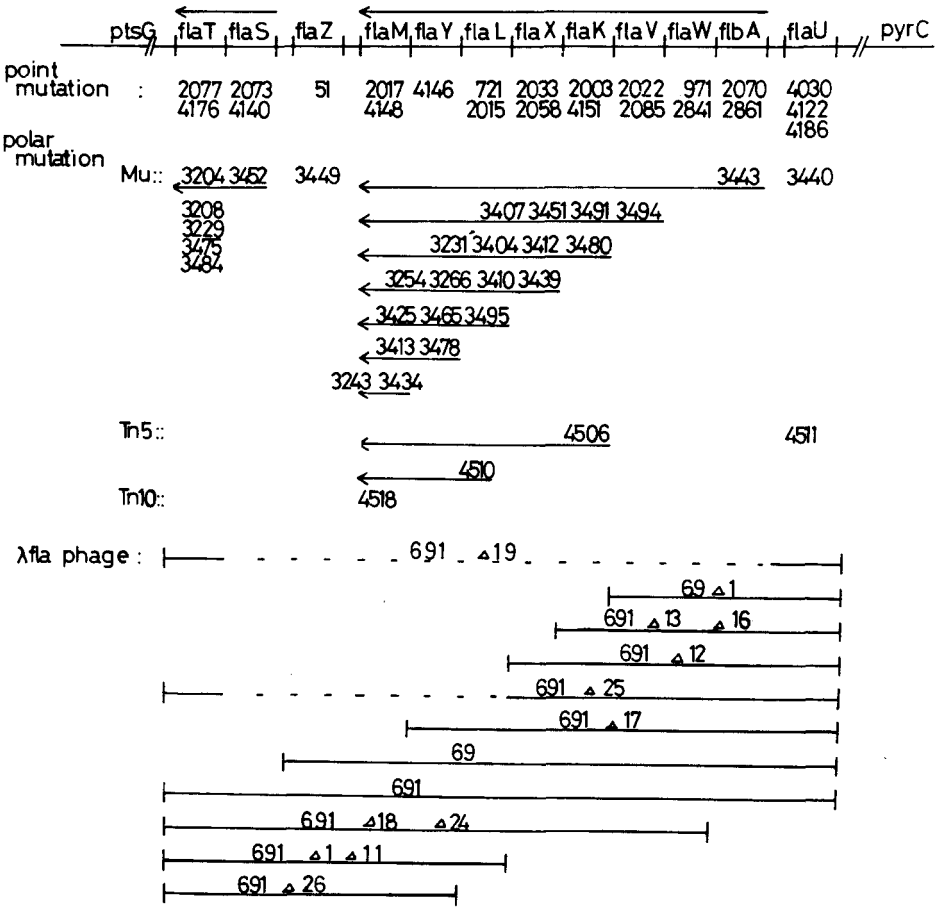


FIGURE 1.—Genetic map of flagellar region I in *E. coli*. This region is located between the *pyrC* and *ptsG* loci. Map locations and functional defects of point and polar mutations studied in this work are indicated by allele numbers and are based on data from complementation tests with P1 phage. Polar mutations are described by arrows that show functional defects. The *E. coli* material present in various λ*fla* transducing phages is indicated by the solid lines. λ*fla* end points are shown by vertical lines. The transcription sequences are shown by arrows above the gene symbols.

two cistrons. They also found that strain YK2078, which was previously thought to have a mutation in the *flaS* gene, was actually a double mutant with mutations in *flaS* and another *fla* gene. This suggested the existence of additional *fla* genes in region I; therefore, new flagellar mutants were isolated and analyzed. We examined 63 spontaneous, 58 Mu phage-induced and four transposon-induced Fla⁻ mutants for complementation by λ*fla691* (KOMEDA *et al.* 1977). The results of reciprocal transductional crosses with the spontaneous Fla⁻ mutants are summarized in Figure 1. The complementation behavior of flagellar mutants indicated the existence of six additional *fla* (*flb*) genes resulting in a total of twelve *fla* cistrons in region I. These complementation groups were designated *flaU*,

*flbA**, *flaW*, *flaV*, *flaK*, *flaX*, *flaL*, *flaY*, *flaM*, *flaZ*, *flaS* and *flaT*. Careful re-examination of mutant crosses done by KOMEDA, SILVERMAN and SIMON (1977) revealed complementation between strains with *flaK* mutations. These mutations were divided into two cistrons, *flaK* and *flaX*. The mutants with defects in the *flaK* cistron showed poor, but significant, complementation by those in the *flaX* cistron. This poor complementation suggests an interaction between these two genes and might well have led to the failure to detect complementation in previous F′ mapping (KOMEDA, SILVERMAN and SIMON 1977). In addition to *flaX*, five more cistrons were identified in region I. These were *flaU*, *flaW*, *flaY*, *flaZ* and *flbA*. The mutants in *flaU* or *flaZ* formed busy swarms; a minor fraction of the cells could swim and showed spotty development of colonies from the inoculated area. This phenotype was specific for these two cistrons, and even null-phenotype mutants showed this leakiness for flagellar synthesis (as described below). In earlier work, the "busy" mutants were not included in complementation analysis. While the mutation in strain MS971 was previously assigned to *flaV* by λ-*fla* transduction, P1-mediated transduction revealed that it belonged to *flaW*. Strain MS51 belonged to another new complementation group that defines *flaZ*.

Fifty-eight Mu-lysogenic mutants with defective flagellar functions were examined by transduction with P1 phage grown on each spontaneous Fla⁻ mutant. Figure 1 also shows the results of some of the complementation tests. Complementation with Mu-induced mutants showed polarity effects; that is, the Mucts phage or Mud(Ap^r, *lac*) insertion affected the expression of more than one gene. Tn-induced mutants were also shown to have multiple *fla* gene defects in the complementation test (Figure 1). Previous work had defined only one operon in this region (KOMEDA, SILVERMAN and SIMON 1977). Careful examination of the polarity of these mutations revealed the existence of complementation between mutants with defects in the *flaS-flaT* operon and mutants with defects in the *flbA* operon. Thus, *flaS* and *flaT* comprise a separate operon. The *flaU* and *flaZ* cistrons were shown to consist of independent operons. Defects in the Mu-induced mutant YK3440 and the Tn5-induced mutant YK4511 were assigned to the *flaU* cistron. While they should have the null phenotype, they made bushy swarms typical of other mutants with *flaU* defects on motility agar at 30°. The Fla⁻ mutant YK3449, *flaZ*::Mud(Ap^r, *lac*), also had the same leaky phenotype for flagellar synthesis. Mutations in *flaU* or *flaZ* did not show any polar effects on the expression of the other cistrons. When strain MS51 (mutant in *flaZ*) was used as donor in P1 transductions, good complementation (relative to the wild-type donor) was observed in tests with strains carrying *flaS*, *flaT* and *flaU* mutations. However, complementation between strain MS51 and one of the mutants in the *flbA* operon was poor relative to the above tests. This fact may suggest an interaction of the *flaZ* and *flbA* operon gene products. The complementation analysis with strains carrying polar mutations clearly showed

* Since the definition of new *fla* genes exhausted the letters available for nomenclature using the genotype symbol *fla*, a new genotype symbol for the Fla⁻ phenotype was suggested by B. BACHMANN. This symbol is *flb* and is equivalent to *fla*.

the gene orders (*flbA-flaW*)-*flaV-flaK-flaX-flaL-flaY-flaM* and *flaS-flaT* (Figure 1). (The order of the genes in parenthesis was not apparent from this analysis.)

The physical orientation of these operons was examined by complementation analysis with deletion derivatives of λ*fla69* and λ*fla691* (Figure 1). The simplest explanation of the result is that the first three genes have the order: *flaU-flbA-flaW*. Complementation tests with pLC strains that mobilize hybrid ColE1-*fla* plasmids supported this order (data not shown). In summary, these results indicate the existence of four operons. The gene order is: *flaU*; $\overrightarrow{flbA, flaW, flaV}$, $\overrightarrow{flaK, flaX, flaL, flaY, flaM}$; *flaZ*; $\overrightarrow{flaS, flaT}$. The arrows above the gene symbols show the direction of transcription.

Complementation analysis of region II flagellar mutants: According to SILVERMAN and SIMON (1977a), λ*fla22* has *flaI*, *motA*, *motB* and part of the *cheA* gene. λ*fla52* has the entire "mocha" operon (*motA*, *motB*, *cheA*, *cheW*), *tar* (*cheM*) and *cheX* (Table 2). Therefore, Mot⁻ mutants are complemented by both λ*fla22* and λ*fla52*. It was thought that those mutants that were complemented by λ*fla22*, but not by λ*fla52*, were mutants with *flaI* defects. In addition to mutants with *flaI* defects, we found other mutants that were complemented by λ*fla22* but were complemented poorly by λ*fla52*. The number of trails (the measure of complementation) formed with λ*fla52* transduction was about one-tenth that with λ*fla22*. This class of mutants had a Fla⁻ phenotype and were not agglutinated by anti-flagella antibody.

We collected those mutants that were complemented by λ*fla22*. The result of the complementation analysis of these mutants is described in Figure 2. The P1-mediated complementation tests clearly indicate the existence of an operon

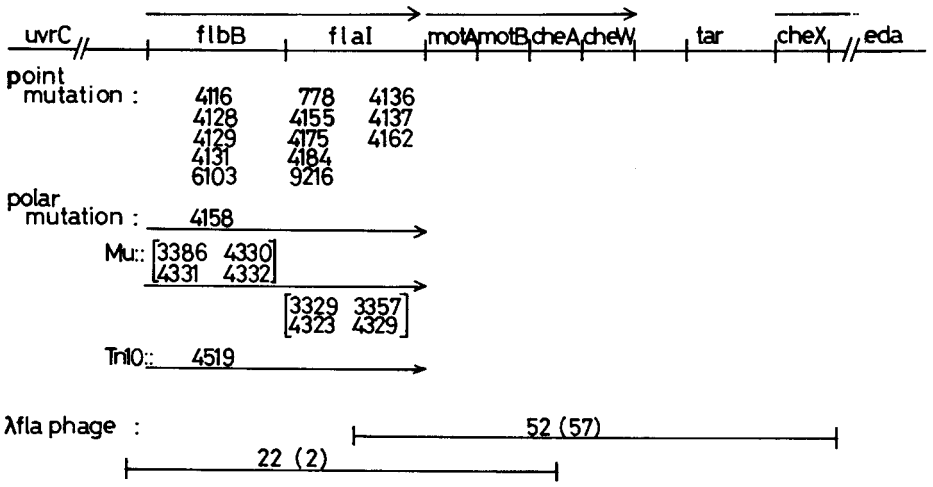
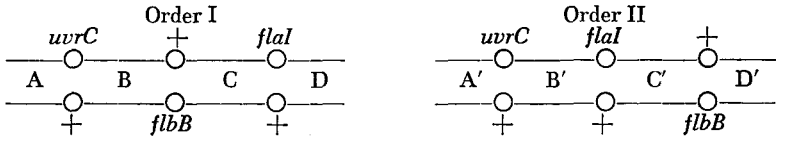


FIGURE 2.—Genetic map of part of flagellar region II in *E. coli*. This region is located between the *eda* and *uvrC* loci. The results are based on data from this study. Symbols are the same as in Figure 1.

TABLE 3

Three-point crosses among uvrC, flbB *and* flaI *mutations*

Donor	Recipient	Selective marker	Resulting characteristics *fla*	*flb*	*uvrC*	No. of transductants	Minimal number of crossovers required Order I	Order II
MS5026	YK4116	*flb*+	+	+	—	18	2(A,C)	4(A′,B′,C′,D′)
(*flaI, uvrC*)	(*flbB*)		+	+	+	30	2(B,C)	2(C′,D′)
YK4116	MS5026	*fla*+	+	+	—	18	2(C,D)	2(B′,C′)
			+	+	+	0	4(A,B,C,D)	2(A′,C′)



containing *flaI* and another gene next to it. We designated the latter as *flbB*. Strains with *flaI* mutations were found in the class of Fla⁻ mutants that were complemented by both λ*fla22* and λ*fla52* (but poorly by the latter phage). Strains YK4136, YK4137 and YK4162 are examples. Other strains with *flaI* defects were not complemented by λ*fla52*. Therefore, the hybrid phage λ*fla52* may have only part of the *flaI* gene. This kind of trail formation has been often observed in λ*fla*-mediated transduction. One possible interpretation of this is a gene-dosage effect of the *fla* genes coded on λ*fla* phages. Complementation analysis with Mu- and Tn-induced mutants showed that the operon was transcribed from *flbB* to *flaI* (Figure 2). The orientation of these genes was examined by three-point crosses between mutants with *uvrC*, *flbB* and *flaI* defects. Table 3 clearly shows the order to be *uvrC-flbB-flaI*. This result is in agreement with the complementation observed in the cross between some mutants with *flaI* defects and λ*fla52*. Hybrid λ*fla52* probably has the distal part of the *flaI* gene. These results agree with the polar effect of the deletion in mutant MS1967 described previously by SILVERMAN and SIMON (1974a). Of the strains originally classified by SILVERMAN and SIMON (1973a) as having *flaI* defects, strain MS6103 is now classified as having a *flbB* defect. This strain was analyzed by λ*fla22* complementation and had not been analyzed by F′ complementation (SILVERMAN *et al.* 1976).

Region III flagellar mutants: Region III Fla⁻ mutants were identified as those mutants that could be complemented by λ*fla#36* phage. These were analyzed by P1 phage-mediated transduction. Figure 3 shows the result of this analysis, which confirmed the results of the genetic analysis performed by SILVERMAN and SIMON (1973a). A novel mutant, strain YK4117, was also isolated. This mutant complemented mutants with *flaA* defects when used as donor, but did not show complementation as recipient in crosses with strains with *flaA* defects. The mutation in this strain was included in the operon starting with *flaA* as shown in Figure 3. Strain YK4020 carrying a multigene defect could not be complemented by strain YK4117, but could be complemented by mutants with other *flaA* defects. Since strain YK4117 might contain a mutation defining a

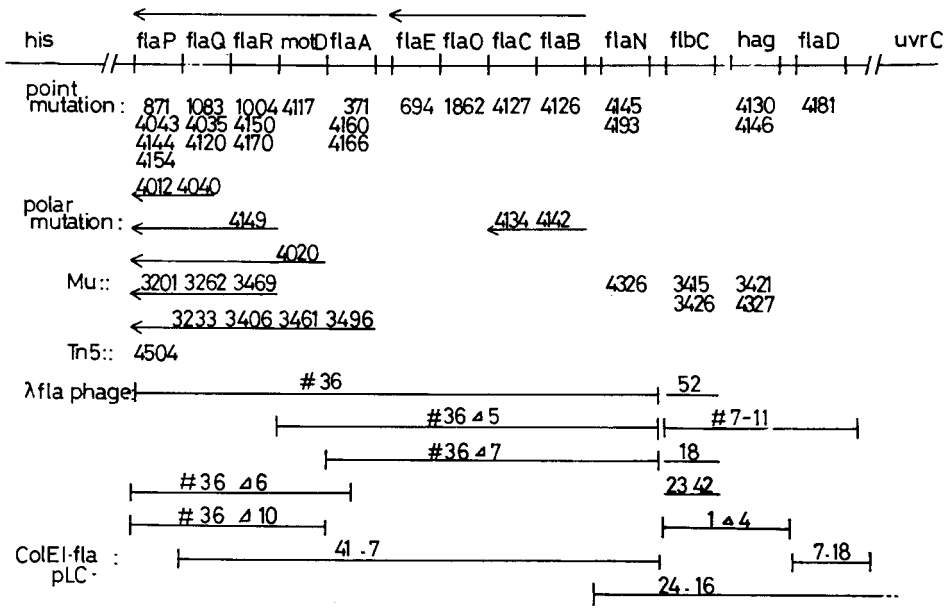


FIGURE 3.—Genetic map of flagellar region III in *E. coli*. This region is located between the *uvrC* and *his* loci. The results are based on data from this study. The *E. coli* material present in ColE1-*fla* hybrids (pLC strains) is indicated by the solid lines, with endpoints indicated by vertical lines. Other symbols are the same as in Figure 1.

new cistron, we tested complementation between λ*fla#36* derivatives carrying deletions in this region and the various mutants described above. The result suggested the existence of a new cistron (Figure 3). Strain YK4117 was shown to have the Mot⁻ phenotype. The cells were agglutinated by anti-flagella antibody, but could not move in L broth or on motility agar plates. Therefore, we designated this cistron as *motD*. We interpret the peculiar complementation of strain YK4117 as resulting from interaction with the *flaA* gene product, and the complementation tests with λ*fla#36* derivatives suggested the existence of a new cistron.

We also examined Mu- and Tn-induced mutants (Figure 3). SILVERMAN and SIMON (1973b) suggested the order: *flaA-flaP-flaQ-flaR*. Recently, H. KONDOH proposed a different order (personal communication). A Tn*5*-induced *flaP* mutant (strain YK4504) did not show a polar effect on the expression of *flaQ* and *flaR*. CLARKE and CARBON's (1976) plasmid pLC41–7 was found to carry *flaN*, *flaB*, *flaC*, *flaO*, *flaE*, *flaA*, *flaQ* and *flaR*, but not *flaP* activity. Taking into account the complementation behavior of strains YK4012 and YK4040 (Figure 3), we propose the order *flaA-flaR-flaQ-flaP*. The *flaP*, *flaQ* and *flaR* mutants showed a very leaky phenotype, *i.e.*, bushy swarm formation on motility agar. This phenotype might have caused confusion in the interpretation of previous complementation behavior. Strain YK4504, like other mutants

carrying *flaP*, *flaQ* or *flaR* defects, appears to have a leaky phenotype, although it should have a null phenotype.

In a collection of Mud(Ap^r, *lac*)-induced Fla$^-$ mutants, a new class of mutants was identified. They were complemented by λ*fla1*Δ4 and its derivatives (see Table 2). λd*fla#7–11* also complemented these mutants. Previous work showed that the difference between λd*fla#7–11* and λ*fla1*Δ4 was the existence of *flaD* on λ*fla#7–11* (Table 2). During the course of the isolation of λ*fla52*, the DNA segment that was on the *his* proximal side of the *hag* gene (see Figure 1 of SILVERMAN *et al.* 1977) was retained. λ*fla#36* infection did not cause these mutants to form trails. Since λ*fla1*Δ4 does not have *flaD*, the mutations in the above class of mutants must be located very close to the *hag* gene. Three-point crosses were performed between strains MS987 (*hag*, *uvrC*) and YK3415, a representative of the strains with the putative new *hag*-linked mutations.

Table 4 shows that the mutation was located on the side of the *hag* gene distal from the *uvrC* gene. Therefore, the genetic tests suggest that these Fla$^-$ mutants are located between *hag* and *flaN*. In order to confirm these results, we isolated a series of Fla$^-$ mutants from strains YK3415 and YK3426 by temperature induction (41°) of the Mu prophage. Strains YK3415 and YK3426 had Mud(Ap^r, *lac*) phage inserted in the presumptive *fla* gene. The Mu phage had temperature-sensitive immunity and could kill the cells by induction at high temperature (41°). Survivors of Mu induction are those cells that, as a result of imprecise excision, lose some of the Mu genes required for the killing function at high temperature. Survivors were also expected to lose some of the chromosomal genes simultaneously. All of the Lac$^-$ heat survivors examined (100 clones from each mutant) showed a stable Fla$^-$ phenotype when they were plated on motility plates. The *fla* mutations in 20 survivors of each parent were analyzed by P1 transduction.

The result is shown in Table 5. Some survivors (for example, #5 and #7 in YK3415) were complemented in crosses by all the Fla$^-$ mutants examined. Others had defects in known *fla* genes. The simplest explanation of the results

TABLE 4

Three-point crosses among flbC, hag *and* uvrC

Donor	Recipient	Selective marker	Resulting characteristics *hag*	*flb*	*uvrC*	No. of transductants	Minimal number of crossovers required Order I	Order II
YK3415	MS987	*hag*+	+	+	—	31	2(B,C)	2(A′,B′)
(*flbC*)	(*hag*, *uvrC*)		+	+	+	17	2(B,D)	4(A′,B′,C′,D′)
MS987	YK3415	*flb*+	+	+	—	2	4(A,B,C,D)	2(B′,D′)
			+	+	+	32	2(A,B)	2(B′,C′)

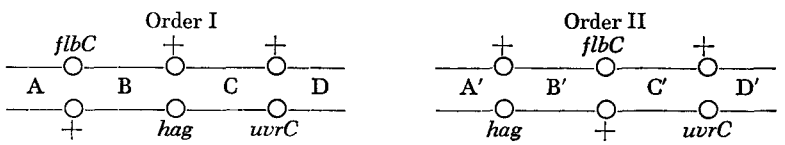


TABLE 5

Complementation of the heat survivors of flbC::*Mud mutants*

Donor	4181 (*flaD*)	4130 (*hag*)	4193 (*flaN*)	4126 (*flaB*)	4127 (*flaC*)	4112 (*flaO*)	4105 (*flaE*)	4160 (*flaA*)
Survivors from YK3415								
#1	+	+	—	—	—	—	—	—
#3	+	+	—	—	—	—	—	—
#4	+	+	+	+	+	+	+	+
#5	+	+	+	+	+	+	+	+
#6	+	+	—	—	—	—	+	+
#7	+	+	+	+	+	+	+	+
#9	+	+	—	—	—	+	+	+
Survivors from YK3426								
#1	+	+	—	—	—	—	—	—
#4	+	+	+	+	+	+	+	+
#5	+	+	+	+	+	+	+	+
#9	+	+	—	—	—	—	—	—

in Table 5 is that the Mu phage was originally located in a gene between *flaN* and *hag* and that the deletions in the survivors started from this point. The heat-surviving clone #5 of strain YK3415 or YK3426 was also shown to be complemented by the λ*fla* phages that made trails on strains YK3415 and YK3426. The complementation behavior with CLARKE and CARBON's plasmid also supported this argument (Figure 3). Strain pLC 7–18 complemented the *flaD* mutants, and strain pLC 24–16 complemented the *flaD*, *hag* and *flaN* mutants. While strain pLC 24–16 complemented strains YK1101 (#5 heat-surviving clone from strain YK3415) and YK1102 (#5 heat-surviving clone from strain YK3426), strain pLC 7–18 did not show complementation with these mutants. These results lead us to conclude that a previously unidentified gene exists between *flaN* and *hag*. We propose that it be designated *flbC*. Complementation analysis with Mu-induced mutants carrying *flaN*, *flbC* or *hag* defects showed that these three genes do not comprise one operon (Figure 3).

DISCUSSION

Figure 4 identifies the flagellar genes on the genome of *E. coli* K12 and includes the eight newly identified genes defined in this study. When the *fla* genes of *E. coli* were compared with those of *S. typhimurium*, KUTSUKAKE *et al.* (unpublished) found homology with respect to location and function, except in a few instances where no homologous gene in *E. coli* had been identified. This study identifies these "missing" *fla* genes in *E. coli* and identified one more gene (*flbC*), which has not yet been found in *S. typhimurium*. Genetic study on the *fla* genes was initiated in Salmonella (STOCKER 1956), and the extensive genetic analysis performed by YAMAGUCHI *et al.* (1972) has extended the findings and

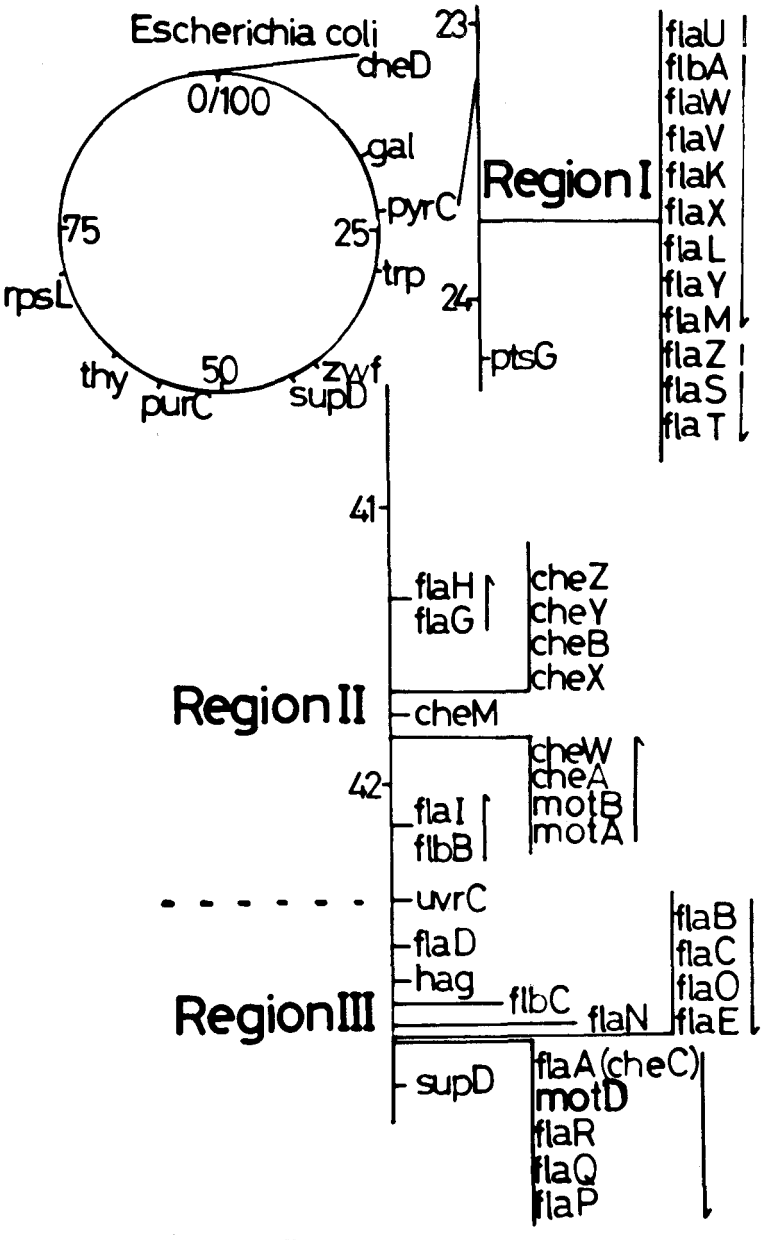


FIGURE 4.—Map of the *E. coli* flagellar genes.

led to a thorough understanding of the genetic architecture of flagellar genes. The comparative work performed by KUTSUKAKE *et al.* (unpublished) also suggested a search to find more *fla* genes in *E. coli*.

In region I, 12 *fla* genes were identified. Previous work performed with F′-*fla* in this region revealed only six genes (KOMEDA, SILVERMAN and SIMON 1977).

We have subdivided one of these (*flaK*) and identified five more genes. There are several reasons for the underestimation of the number of *fla* genes in previous studies, which employed F′-*fla* elements in complementation tests. F′ transfer of the element (F′1005) was very poor, and we could not construct F′1005 carrying *fla* mutations by recombination. Accordingly, *fla* mutations of F′1005 were collected from strain YK110, which turned out to have a chromosomal deletion of *flaV* through *flaT* (data not shown) and which has F′1005. This was the reason why the previous work failed to define the genes *flaU* through *flaW* (see Figure 4).

We employed an additional approach, P1-mediated transduction. This method enabled us to perform extensive reciprocal complementation tests between pairs of region I Fla$^-$ mutants. Some crosses were characterized by poor complementation. This fact might suggest some interactions between structural components. This interaction of gene products may have led to difficulty in interpreting complementation behavior in previous studies.

In region II, *flbB* was identified and located adjacent to *flaI*. It was shown by analysis of Mu- and Tn-induced Fla$^-$ mutants to be a member of an operon transcribed from *flbB* to *flaI*. So far, cAMP is the only known regulator that controls flagellar formation. A cAMP-independent mutation, *cfs* (*c*onstitutive *f*lagellar *s*ynthesis) was mapped near *flaI* by deletion analysis, and regulation studies indicated that *flaI* may control the expression of other flagellar genes (SILVERMAN and SIMON 1974b). Strain MS6103, which actually has a *flbB* defect, has been used for flagellar gene product analysis (SILVERMAN *et al.* 1976). It can not support the synthesis of many flagellar proteins programmed by λ*fla* phages. Our results suggest that, since *flaI* and *flbB* comprise one operon, *cfs* may be a promoter mutation that affects expression of both genes. Thus, both the *flaI* and the *flbB* gene products may regulate in a positive fashion the expression of all the other flagellar genes. We are currently trying to map the *cfs* mutation with respect to these two genes.

In region III, *flbC* was identified and located between *flaN* and *hag*. Genetic analysis performed with λ*fla* phages, P1 phage or CLARKE and CARBON's plasmids clearly determined its location. No missense mutation in this gene has been isolated in this study. The *flbC* gene was also shown to be a separate transcriptional unit. The genes in the *flaA* operon have been reordered: *flaA-flaR-flaQ-flaP*. This order conforms to the gene order of the homologous Salmonella region (KUTSUKAKE, unpublished). Strain YK4117 (*motD*) may define a new cistron between *flaA* and *flaR*.

KONDOH and OZEKI (1976) defined a new gene *flaX*, between *hag* and *flaXI*. We could not find any mutants with defects located in this region. They used a straight flagellar filament mutant as a representative mutant in the *hag* gene. We noticed that straight filament mutants are not good recipients for P1-mediated transduction, or even for λ*hag*-mediated transduction. When these mutants were used as donors in P1 transduction, complementation (trail formation) was quite poor. Thus, the straight filament mutants behave in complementation tests as strains with dominant mutations. We suggest, therefore, that the *flaX* gene is

really an allele of the *hag* gene, as suggested in Table 3 of KONDOH and OZEKI (1976).

We now have identified 28 flagellar genes for construction of the flagellar apparatus and at least 10 genes involved in the function of the flagella. Supposing there are 3000 genes in *E. coli* K12, those genes responsible for flagellar formation and function comprise more than 1% of the total. The number of flagellar genes nearly corresponds to that of ribosomal genes. There must be considerable evolutionary advantage in the existence of flagella, so that we expect there to be a complex means of regulation. Also, we have found sequential expression of flagellar genes, and the gene expression is controlled positively (KOMEDA, unpublished).

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LITERATURE CITED

CASADABAN, M. J., 1976 Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104:** 541–555.

CLARKE, L. and J. CARBON, 1976 A colony bank containing synthetic Col E1 plasmids representative of the entire *Escherichia coli* genome. Cell **9:** 91–99.

FRANKLIN, N. C., 1978 Genetic fusions for operon analysis. Ann. Rev. Genet. **12:** 193–221.

HARAYAMA, S., E. T. PALVA and G. HAZELBAUER, 1979 Transposon-insertion mutants of *Escherichia coli* K12 defective in a component common to galactose and ribose chemotaxis. Molec. Gen. Genet. **171:** 193–203.

HOWE, M. M., 1973 Prophage deletion mapping of bacteriophage Mu-1. Virology **54:** 93–101.

ICHO, T. and T. IINO, 1978 Isolation and characterization of motile *Escherichia coli* mutants resistant to bacteriophage χ. J. Bacteriol. **134:** 854–860.

IINO, T., 1977 Genetics of structure and function of bacterial flagella. Ann. Rev. Genet. **11:** 161–182.

KLECKNER, N., D. F. BARKER, D. G. ROSS and D. BOTSTEIN, 1978 Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics **90:** 427–461.

KOMEDA, Y., T. ICHO and T. IINO, 1977 Effects of *galU* mutation on flagellar formation in *Escherichia coli*. J. Bacteriol. **129:** 908–915.

KOMEDA, Y., K. SHIMADA and T. IINO, 1977 Isolation of specialized lambda transducing bacteriophages for flagellar genes (*fla*) of *Escherichia coli* K12. J. Virol. **22:** 654–661.

KOMEDA, Y., M. SILVERMAN and M. SIMON, 1977 Genetic analysis of *Escherichia coli* K12 region I flagellar mutants. J. Bacteriol. **131:** 801–808.

KONDOH, H. and H. OZEKI, 1976 Deletion and amber mutants of *fla* loci in *Escherichia coli* K12. Genetics **84:** 403–421.

LEDERBERG, J., 1956 Linear inheritance in transductional clones. Genetics **41:** 845–871.

SILVERMAN, M., P. MATSUMURA, R. DRAPER, S. EDWARDS and M. SIMON, 1976 Expression of flagellar genes carried by bacteriophage lambda. Nature (London) **261:** 248–250.

SILVERMAN, M., P. MATSUMURA, M. HILMEN and M. SIMON, 1977 Characterization of lambda *Escherichia coli* hybrids carrying chemotaxis genes. J. Bacteriol. **130:** 877–887.

SILVERMAN, M., P. MATSUMURA and M. SIMON, 1976 The identification of the *mot* gene product with *Escherichia coli*-lambda hybrids. Proc. Natl. Acad. Sci. U.S. **73:** 3126–3130.

SILVERMAN, M. and M. SIMON, 1973a Genetic analysis of flagellar mutants in *Escherichia coli*. J. Bacteriol. **113:** 105–118. ——, 1973b Genetic analysis of bacteriophage Mu-induced flagellar mutants in *Escherichia coli*. J. Bacteriol. **116:** 114–122. ——, 1974a Positioning flagellar genes in *Escherichia coli* by deletion analysis. J. Bacteriol. **117:** 73–79. ——, 1974b Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J. Bacteriol. **120:** 1196–1203. ——, 1977a Identification of polypeptides necessary for chemotaxis in *Escherichia coli*. J. Bacteriol. **130:** 1317–1325. ——, 1977b Bacterial flagella. Ann. Rev. Microbiol. **31:** 397–419.

STOCKER, B. A. D., 1956 Abortive transduction of motility in *Salmonella*; a non-replicated gene transmitted through many generations to a single descendant. J. Gen. Microbiol. **15:** 575–598.

SUZUKI, T., T. IINO, T. HORIGUCHI and S. YAMAGUCHI, 1978 Incomplete flagellar structures in nonflagellate mutants of *Salmonella typhimurium*. J. Bacteriol. **133:** 904–915.

YAMAGUCHI, S., T. IINO, T. HORIGUCHI and K. OHTA, 1972 Genetic analysis of *fla* and *mot* cistrons closely linked to *H1* in *Salmonella abortusequi* and its derivatives. J. Gen. Microbiol. **70:** 59–76.

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