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Genetic Analysis of Flagellar Mutants in *Escherichia coli*

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Flagellar mutants in *Escherichia coli* were obtained by selection for resistance to the flagellotropic phage χ. F elements covering various regions of the *E. coli* genome were then constructed, and, on the basis of the ability of these elements to restore flagellar function, the mutations were assigned to three regions of the *E. coli* chromosome. Region I is between *trp* and *gal;* region II is between *uvrC* and *aroD;* and region III is between *his* and *uvrC*. F elements carrying flagellar mutations were constructed. Stable merodiploid strains with a flagellar defect on the exogenote and another on the endogenote were then prepared. These merodiploids yielded information on the complementation behavior of mutations in a given region. Region III was shown to include at least six cistrons, *A, B, C, D, E,* and *F*. Region II was shown to include at least four cistrons, *G, H, I,* and *J*. Examination of the phenotypes of the mutants revealed that those with lesions in cistron *E* of region III produce "polyhooks" and lesions in cistron *F* of region III result in loss of ability to produce flagellin. Mutants with lesions in cistron *J* of region II were entirely paralyzed (*mot*) mutants. Genetic analysis of flagellar mutations in region III suggested that the mutations located in cistrons *A, B, C,* and *E* are closely linked and mutations in cistrons *D* and *F* are closely linked.

Mutants with altered flagellar apparatus can be prepared by selecting clones resistant to the flagellotropic phage χ. Most of these clones are nonmotile and are either paralyzed (possess flagella but have no capacity for translational motion), nonflagellated, or exhibit the polyhook phenotype (possess abnormally terminated hook structures and show rapid spinning motion). Most nonmotile χ-resistant mutants are of the nonflagellated variety. The mutations can be grouped according to function by analyzing the complementation behavior of pairs of mutations in partial diploids. Extensive analysis of the complementation behavior of flagellar mutants in *Salmonella typhimurium* has been carried out by using P22-mediated abortive transduction (8, 9, 11, 14, 23). This analysis allowed the definition of at least 15 cistrons that are involved in flagella formation. In studies with *Escherichia coli*, P1-mediated abortive transduction (1, 2) has yielded information concerning the genetic organization of the flagellar system, particularly the distribution of mutations affecting motility and chemotaxis. However, there has not been an extensive complementation analysis of nonflagellated mutants in *E. coli*. Such an analysis would be useful in further defining the functions necessary for the assembly and activity of bacterial flagella.

In this investigation F elements were useful both in locating a given flagellar defect on the *E. coli* chromosome and in performing complementation analysis. Flagellar mutations were located in three regions of the *E. coli* chromosome; region I, between *trp* and *gal;* region II, between *uvrC* and *aroD*; and region III, between *his* and *uvrC*. These regions were first described by Adler and Armstrong (3) in connection with studies of paralyzed and chemotaxis mutants of *E. coli*. This paper extends their approach. We have defined at least six cistrons in region III, *A, B, C, D, E,* and *F*, and at least four cistrons in region II, *G, H, I,* and *J*. Examination of the phenotypes of mutants in each of the cistrons revealed that all of the mutations in cistron *E* result in the production of "polyhooks." This cistron is referred to as *flaE* (18). Mutations in cistron *F* affect the production of flagellin and thus correspond to the *hag* locus (20). All of the strains carrying mutations that were located in cistron *J* have the paralyzed phenotype. Thus, cistron *J* corresponds to the *mot* locus. Strains carrying muta-

tions in all of the other cistrons had no observable flagellar filament structures. This phenotype is characteristic of *fla* mutants (23). Linkage analysis by P1-mediated transduction confirmed the assignment of region III mutations to a location between *his* and *uvrC* and indicated two gene clusters, *flaA*, *B*, *C*, *E* and *flaD* and *hag* in this region.

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MATERIALS AND METHODS

Media. Tryptone broth contained per liter of distilled water: tryptone (Difco), 10 g; NaCl, 5 g; and thymine, 0.1 g. L broth contained per liter of distilled water: tryptone, 10 g; NaCl, 10 g; yeast extract (Difco), 5 g; glucose, 2 g; and thymine, 0.1 g. Glucose was added aseptically after autoclaving. L agar plates were prepared by adding 1.5% agar (Difco) to L broth. Motility plates were prepared by adding 0.35% agar to tryptone broth.

Minimal medium contained per liter of distilled water: K_2HPO_4, 11.2 g; KH_2PO_4, 4.8 g; $(NH_4)_2SO_4$, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; $Fe_2(SO_4)_3$, 0.5 mg; glucose, 5 g; and thiamine, 1 mg. The $MgSO_4 \cdot 7H_2O$, glucose, and thiamine were added aseptically after autoclaving. Amino acids and thymine, if required, were added to a final concentration of 100 mg/liter. Minimal motility plates were prepared by substituting glycerol for glucose and adding 0.35% agar to minimal medium. Minimal agar plates were prepared by adding 1.5% agar to minimal medium.

Bacteria. The *E. coli* K-12 strains are listed in Table 1 with their genotypes and derivation. The mutants described in this study were derived from strain MS1350. Strain MS1350 was prepared by M. Silverman in M. Simon's laboratory from a K-12 strain, AB1884, obtained from J. Adler. The strain was made Lac$^+$, Pro$^+$, Gal$^+$, Thr$^+$, and Leu$^+$ by conjugation with strain KL96, and then Trp$^-$ by bromodeoxyuridine auxotroph selection (4). The *galU* marker was introduced by P1 transduction from W4597 by selecting for Trp$^+$ Gal$^-$ recombinants. Thy$^-$ strains were obtained by trimethoprim treatment (19). The absence of suppressors was determined by the inability of phage T4 amber B22 to form plaques on this strain.

Two antigenic variants of the flagellar antigen (Hag) were used in this study. Hag 207 refers to the flagellar filament derived from *E. coli* strain MS1350. *hag-207* refers to the allele responsible for the production of this antigen. Antisera from rabbit no. 207 immunized with purified flagellar filaments reacted with strains MS1350 and MS1275. However, these antisera did not react with antigen derived from strain MS1032 or MS1276. Hag 208 refers to the flagellar filament derived from *E. coli* strains MS1032 and MS1276 which were selected for their ability to swim through motility agar containing antiserum 207. Antisera from rabbit no. 208 immunized with purified flagellar filaments from MS1032 reacted with the flagellar antigens from strains MS1032 and MS1276 but not with the flagellar antigens derived from strain MS1350 or MS1275.

Isolation of mutants. Flagellar mutants were selected for their resistance to the flagellotropic phage χ (13, 15) after mutagenesis with ethyl methanesulfonate (EMS). The procedure of M. Wright (22) was used for mutagenesis, except minimal medium was used and 0.05 ml of EMS was added to 2.5 ml of cell concentrate. Phage resistance selection was accomplished on L agar plates with an overlay of soft agar consisting of a mixture of 2.5 ml of motility agar plus 0.1 ml of exponential-phase cells grown to allow the mutations to segregate and 0.1 ml of χ phage for a final multiplicity of infection of approximately one. Survivors were streaked twice on minimal agar plates and then tested for motility. Glucose must be excluded from the medium used to cultivate flagellated cells since the synthesis of these structures is subject to catabolite repression (24). Mutants suppressible by Φ80d Su_{III}^+ transducing phage (a gift from J. Abelson) were classified as amber mutants. A limited number of mutants were saved from each mutagenesis. Only those mutants that were clearly distinguishable as having arisen independently (by differences in the locations of their lesions and by their response to amber suppressors) were saved. Mutant strains were given allele numbers and strain designations. The strain designation was derived from the allele numbers by adding the prefix MS.

Rec$^-$ selection. *recA* recipients were required for F selection and complementation analysis. *recA* selection took advantage of the close linkage of *thy* and *recA*. An Hfr that transfers *recA* early, JC5072, was mated with a *thy* recipient, and Thy$^+$ recombinants were selected. Only the small Thy$^+$ recombinant colonies were *recA* as judged by their inability to support recombination for the *his*$^+$ marker, mediated either by P1 transduction or Hfr transfer.

F selection. F elements were generated by the method of B. Low (12) by using the Hfr KL96 which donates *his*$^+$ as the proximal marker into a *his recA* recipient. Episomes bearing the *his aroD* region were sought, and several useful ones were obtained. F1334 has been shown to cover the *his* and *uvrC* locus. F *his*$^+$, *uvr*$^+$ transfer was tested by conjugation with a *rec*$^+$, *his*, *uvrC* recipient with selection for His$^+$ exconjugants. His$^+$ clones were then scored for inheritance of *uvr*$^+$ by spreading the clones on an L agar plate and exposing the surface to a ultraviolet light (UV) dose of about 400 ergs/mm^2. UV resistance could be ascertained after overnight incubation of the plate. One F *his*$^+$, *uvr*$^+$, *zwf*$^+$ element, F1338, was isolated. *zwf*$^+$ transfer could be measured by conjugation with strain MS1017, which carried the *his*, *zwf*, *pgl* markers. This strain was constructed by selecting for Fla$^+$ recombinants after mating strain DF2001, which was HfrC *zwf*, with strain SA197, which carried *his*, *pgl* (*blu*), *fla*. About 50% of the Fla$^+$ recombinants carried the *zwf*, *pgl*, *his* genes. If the *zwf*$^+$ marker is transferred to strain MS1017 by conjugation and subsequent selection for His$^+$, the strain acquires the His$^+$, Zwf$^+$, Pgl$^-$ phenotype and can be identified by the "blu" test (10).

Mucoid merodiploid strains. Strains diploid in the *his uvrC* region are extremely mucoid, and the

TABLE 1. *Bacterial strains*

Strain	Mating type	Relevant markers	Source
KL96	Hfr	*thi*, λ^-	B. Low
AB1884	F^-	*thi, thr, leu, pro, his, argE, str, lac, gal, ara, xyl, mtl, hag-207, uvrC*	J. Adler
MS1275	F^-	AB1884 except *thy*	Trimethoprim treatment of AB1884
MS1276	F^-	MS1275 except *hag-208*	Antibody selection of MS1275
AB2463	F^-	*thi, thr, leu, pro, his, argE, str, lac, gal, ara, xyl, mtl, recA13, hag-207*	D. Kingsbury
MS1032	F^-	AB2463 except *hag-208*	Antibody selection of AB2463
JC5072	Hfr	*thr, ilv, thi, str*$^+$, *spc, recA67*	A. J. Clark
MS1300	F^-	MS1275 except *recA67*	JC5072 → MS1275 for *thy*$^+$ *recA*
MSF1333	F′	F′*his*$^+$, *uvr*$^+$ in MS1300	KL96 → MS1300 for *his*$^+$
JC1553	F^-	*leu-2, his-1, argG, met-1, str, lac-4, malA1, xyl, mtl, recA1*	A. J. Clark
MSF1334	F′	F *his*$^+$, *uvr*$^+$ in JC1553	MSF1333 → JC1553 for *his*$^+$
MSF1336	F′	F *his*$^+$, *uvr*$^+$, *zwf*$^+$ in MS1300	KL96 → MS1300 for *his*$^+$
MSF1338	F′	F *his*$^+$, *uvr*$^+$, *zwf*$^+$ JC1553	MSF1336 → JC1553 for *his*$^+$
DF2001	Hfr	*zwf-2, str*$^+$, *fla*$^+$	J. Abelson
SA197	F^-	*his, blu, str, fla*	J. Abelson
MS1017	F^-	*his, zwf-2, blu, str, fla*$^+$	DF2001 → SA197 for fla$^+$
W4597	F^-	*galU*	J. DeMoss
MS1350	F^-	*uvr, galU, sup*$^+$, λ^-, *str, hag-207, his, thy, argE*	M. Silverman
KL181	F^-	*trp, pyrD, gal, his, str, recA1*, λ^-, *sup*$^+$	B. Low
KLF23	F′	F′*trp*$^+$ in KL181	B. Low
KLF26	F′	F′*trp*$^+$, *pyrD*$^+$, *gal*$^+$ in KL181	B. Low
MS1380	F^-	MS1350 except *his*$^+$, *uvr*$^+$	KL96 → MS1350 for *his*$^+$, *uvr*$^+$

production of this extracellular polysaccharide interfered with the production of flagella to the extent that motility was severely impaired. We do not know the basis for this effect. Mucoid *E. coli* strains produce a capsular polysaccharide, the synthesis of which depends upon uridine diphosphate-galactose metabolism because *galE* and *galU* mutants cannot produce the polysaccharide (7). We introduced the *galU* defect from strain W4597 into our basic strain, MS1350, by P1 transduction and selection for Trp^+ Gal^- recombinants. Derivatives of this strain diploid in the *his uvrC* region were then nonmucoid, and flagellar function was restored although movement of the diploid strain was slower than that of the haploid strains.

Mapping with F elements. Various F elements were used to locate the flagellar mutants on the *E. coli* chromosome. Strains MSF1334 and MSF1338 were mated with *rec*$^+$, *fla* mutant strains derived from strain MS1350 on L agar plates for 6 hr, and then approximately 10^7 cells were transferred with a sterile loop to a minimal agar motility plate which selected His^+ recipients and counterselected the donor by multiple amino acid deprivation. Strain KLF26 was mated in an identical fashion except the donor was counterselected by using 200 mg of trimethoprim per liter in the medium, preventing the growth of Thy^+ cells. Movement of the recipient cells from the zone of inoculation of the mating mixture was taken to indicate transfer of the nondefective flagellar allele.

Complementation analysis. Complementation analysis in the region covered by F1334 and F1338 required the construction of merodiploid strains carrying different flagellar defects on the exogenote and the endogenote. This necessitated the transfer of the flagellar mutations to the episome. The mutation was found to reside often on the F element in *rec*$^+$ merodiploid strains. About 2% of the F *his*$^+$ episomes transferred out of the *rec*$^+$ *fla* recipient into a *his recA* repository strain, JC1553, were shown to possess the mutant character and not a deletion by subsequent mating with the original *rec*$^+$ flagellar mutant and other *rec*$^+$ flagellar mutants. Fla^+ exconjugants (probably recombinants) could be produced in all matings except with the identical *rec*$^+$ *fla* recipient. The episomes appeared stable in a *recA* strain, but deletion in the F element occurred frequently if the episome was carried in a *rec*$^+$ strain.

To test for complementation of flagellar defects, it was necessary to eliminate the possibility of the production of a nondefective genotype that could be formed by recombination. The *recA* marker was therefore introduced into the recipient strains. Various F *his*$^+$ *fla* episomes in strain JC1353 were transferred into *recA fla* recipients by mating in L broth and selecting for *his*$^+$ transfer into the *his* recipient on minimal agar motility plates. Mating was carried out by growing donor and recipient strains in L broth at 37 C to a concentration of 1×10^8 to 2×10^8 cells/ml and then mixing them at a ratio of 1:10. The cultures were shaken gently for aeration during mating and chilled on ice after 60 min. A sterile 1 by 0.5 cm Whatman filter paper strip was soaked in the culture and inserted into a minimal

medium motility agar plate which counterselected the donor and selected for His$^+$ recipient exconjugants. Movement was compared after 6 to 8 hr at 37 C (see Fig. 3).

Rescue of cryptic flagellin pools. The flagellin gene (*hag* locus) has been mapped in region III (3). A method to show which of the cistrons corresponded to the flagellin gene was developed. The method was based on the observation that, when merodiploid strains were constructed with different alleles at the *hag* locus determining antigenically different flagellins, flagella were synthesized with both flagellins in the same filament (Silverman and Simon, *unpublished results*). Thus, if the flagellar defect in region III is not in the *hag* gene, the *hag* gene product, which is cryptic in the haploid cell, should be rescued by an F element covering region III. However, if the flagellar mutation is in the *hag* gene, the *hag* gene product will not become apparent in the diploid. Two different sets of merodiploid strains were constructed: one with region III defects and *hag-207* on the chromosome and a nondefective region III genotype with *hag-208* on the episome; and another set with region III defects and *hag-207* on the episome and the nondefective genotype with *hag-208* on the chromosome. Rescue with the first set, F *fla*$^+$ *hag-208/fla hag-207*, was measured by the prevention of movement of these merodiploids through motility agar containing anti-Hag 207 antibody. Rescue with the second set, F *fla hag-207/fla*$^+$ *hag-208* was measured by complement fixation assay specific for the Hag 207 antigen on whole bacteria. The first set of merodiploids were *galU* strains with which complement fixation analysis was difficult because they interfered with the hemolysis reaction. Complement fixation analysis was performed by the procedure of Wasserman and Levine (21).

Fine mapping. Linkage analysis in region III was attempted to confirm the location of region III mutations between *his* and *uvrC* and to study the organization of the genes in this region. This analysis was performed by P1 transduction selecting for His$^+$ and Uvr$^+$ recombinants. Selection for Uvr$^+$ was accomplished by the method of Armstrong and Adler (3) except that transductants were plated on minimal agar to avoid phage killing of recipients. Because strain MS1350 contains a *galU* lesion, the infection of P1 is blocked (5), and a P1 variant had to be selected that would infect this host. The resulting P1 was virulent, and the multiplicity of infection had to be kept below 0.1 to prevent killing of the transductants. P1kc was obtained from D. Kingsbury.

Electron microscope examination. All mutants in the complementation analysis study were examined with a Phillips 200 electron microscope (18).

Antisera. Antisera against flagellar antigen was prepared as reported elsewhere (18).

RESULTS

Location of flagellar defects. Fla$^-$, Mot$^-$, and polyhook mutants were obtained by χ selection. F elements covering three regions of the *E. coli* chromosome were found to restore the motility of the flagellar mutants. The F elements employed and the flagellar gene regions that they covered are shown in Fig. 1. Region I is between *trp* and *gal;* region II is between *uvrC* and *aroD;* and region III is between *his* and *uvrC*. F1334 and F1338 were constructed in this laboratory. Fine mapping data, presented later, confirmed that F1334 covers only mutations between *his* and *uvrC* and not mutations between *uvrC* and *aroD*. The extent of F1338 is not known, and region II mutants may lie between *uvrC* and *zwf*. On the basis of the ability of these F elements to restore flagellar function, flagellar mutations could be assigned to one of the three regions mentioned. Figure 2 summarizes the assignment of flagellar mutations to chromosomal region I, II, or III. Of 320 mutant strains screened, the lesions in 76 were assigned to region I; in 91 to region II; and in 153 to region III. The motility observed under these conditions probably represented recombinants resulting from F matings since movement due to complementation was poorer and could be distinguished from movement in haploids.

Complementation analysis. In *rec*$^+$ merodiploid strains with the flagellar defect on the endogenote, the defect was often found to appear on the F element. F elements carrying various flagellar defects in region II and III were collected. To study complementation between different flagellar mutations, merodiploid strains were constructed with different flagellar

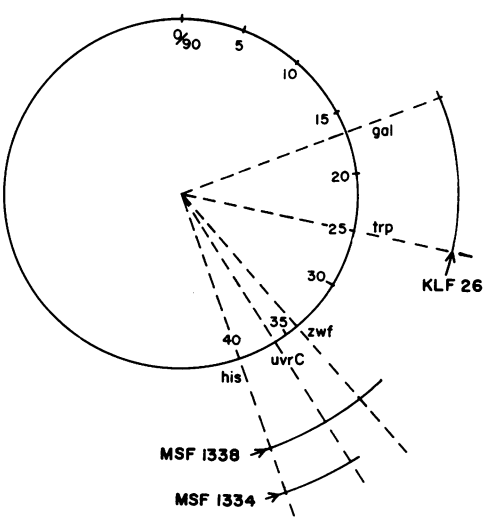

FIG. 1. *F elements in E. coli used in the genetic analysis of flagellar mutations. The arcs represent the region of the E. coli chromosome carried by the F element.*

	mutant											
	41	234	726	912	1083	161	616	797	1105	262	704	952
MSF 1334	+	+	+	+	+	−	−	−	−	−	−	−
MSF 1338	+	+	+	+	+	+	+	+	+	−	−	−
KLF 26	−	−	−	−	−	−	−	−	−	+	+	+
Fla Region	III					II				I		

FIG. 2. *Mapping flagellar mutations with F elements. Symbols: +, flagellar function restored by the F element denoted in left-hand column; −, flagellar function not restored. Mutations were assigned to region I if motility was restored by KLF26, to region II if motility restored by MSF1338, and to region III if motility was restored by MSF1334 and MSF1338.*

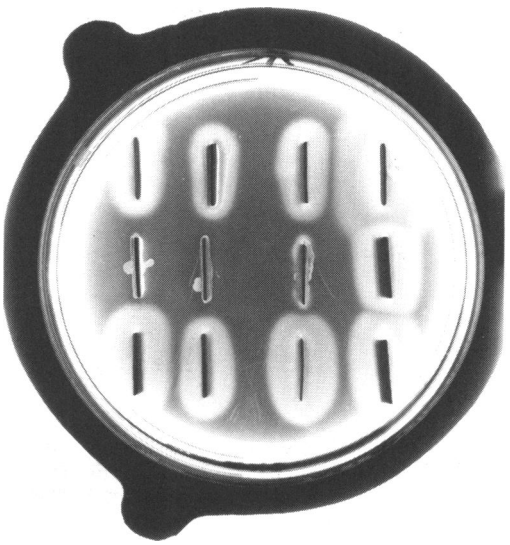

FIG. 3. *Complementation analysis of flagellar mutations. Row 2, column 1 and 2, show no complementation and are scored as* O. *Row 2, column 3 shows poor complementation which is scored as* ⊕. *The remaining merodiploids show good complementation, scored as* +.

defects on the exogenote and endogenote. It was necessary to make the recipient strain *recA* in order to avoid confusion resulting from the production of a nondefective genotype by recombination. The degree of complementation could be determined by observing the movement of the *rec* merodiploid strains on motility agar as in Fig. 3.

Information on the complementation behavior of flagellar mutations obtained in this manner indicated that there are at least four cistrons in region II (Fig. 4). Upon electron microscope examination of mutants with lesions in this region, we found complementation group *J* to consist entirely of paralyzed (Mot$^-$) mutants, that is, mutants that possess flagella which appear normal when examined by electron microscopy but do not function; no translational motion of the bacteria was observed. This cistron will, therefore, be referred to as the *mot* gene.

We found at least six cistrons in region III (Fig. 5). Mutations *fla-775*, *fla-9716* and *fla-107* were the only ones tested that clearly showed membership in two cistrons. Their joint membership may be explained by the fact that all three are amber suppressible and could exhibit polar effects. Strains carrying mutations in cistron *E* were found by electron microscope examination to produce filaments 1 to 2 μm long with a λ of 0.12 μm. These mutants were characterized and are believed to be "polyhook" mutants resulting from the defective termination of the hook region of the flagellum (18). All four group *E* mutants showed the same polyhook phenotype. Region I mutants were also examined by electron microscopy, and all were nonflagellated. Therefore, only *flaE* and *mot* mutants produce filaments external to the cell.

Flagellin has been shown to be a product of a gene in region III (3). Merodiploid strains for region III that produce distinguishable flagellin molecules synthesize flagella composed of both flagellin proteins. Thus, it was reasoned that cryptic *hag* gene expression could be measured in region III mutants by rescuing the function with an F element bearing a nondefective flagellar genotype for region III. The exogenote was prepared by exchange with strain MS1276 which produces an antigenically altered flagellin, Hag 208. The merodiploids prepared and the rescue of the Hag 207 product are shown in Table 2. The presence of Hag 207 antigen prevented the merodiploids from swimming through motility agar containing anti-Hag 207 serum. Rescue was also tested with the region III flagellar mutants on the exogenote in merodiploid derivatives of strain MS1032. The presence of the Hag 207 product was measured by complement fixation analysis specific for Hag 207 flagella on Formalin-fixed whole bacteria (Table 3). We conclude that cistron *F* is the one that is responsible for the production of the flagellin protein. Therefore, the alleles classified as belonging to this cistron *F* define the *hag* locus, and the mutations in cistron *F* will now be referred to as *hag* mutations.

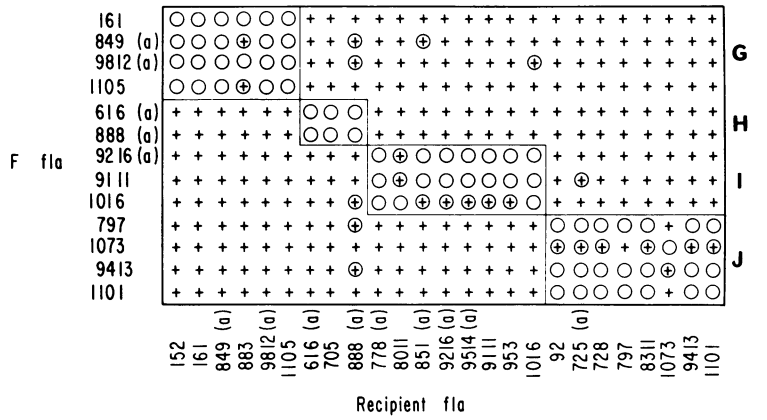

FIG. 4. *Summary of complementation behavior between flagellar mutations in region II. Merodiploid strains were prepared with one flagellar defect from the left column on the exogenote, F1338, and one flagellar mutation from the bottom row on the endogenote. Symbols: +, complementation; ⊕, poor complementation; O, no complementation. (a) refers to mutations found to be amber mutations. Letters in right column denote the cistron.*

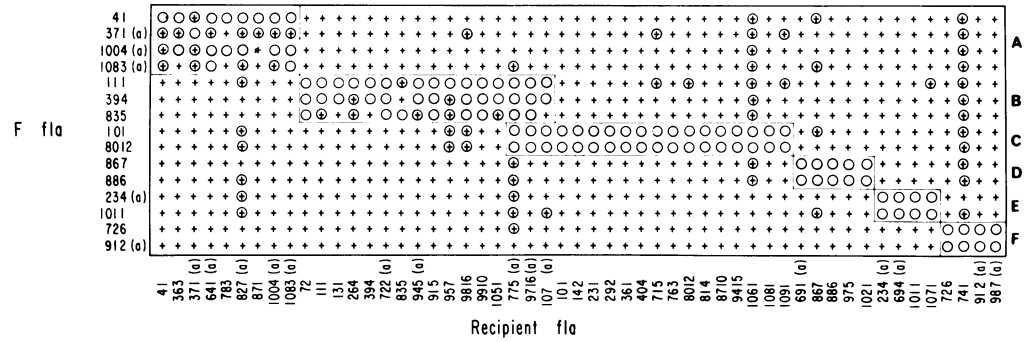

FIG. 5. *Summary of complementation behavior between flagellar mutations in region III. Symbols are the same as Fig. 4: F element is F1334.*

Fine mapping in region III. Since the extent of F1334 beyond *uvrC* was not known except that it did not complement *mot* mutants and did not cover *zwf* (Silverman and Simon, *unpublished results*), it was possible that some mutations covered by this episome were on the side of *uvrC* distal to *his*. Cotransduction of several region III mutations established their location between *his* and *uvrC* (Table 4). This is clear from the poor inheritance of Uvr^+ with the His^+ Fla^+ recombinants. If *uvrC* were between *his* and any of the *fla* mutants, the inheritance of *uvrC* would have been similar to the inheritance of fla^+ with *his*. Three-factor crosses with uvr^+ used as the selected marker related the position of the other cistrons in region III to the mutations discussed in Table 4. Table 5 gives a summary of the three-factor crosses. Although the data do not reveal the order for very closely linked, possibly adjacent genes, they do position all of the cistrons in region III between *his* and *uvrC*. The very low level of recombination to give Fla^+ recombinants for certain crosses, i.e., *flaB* × *flaC*, may be taken as evidence for a close spacing of the genes involved in these crosses. On the other hand, some crosses indicate a gap between certain genes such as crosses between genes *flaA*, *B*, *C*, and *E* and genes *flaD* and *hag*. Cotransduction frequencies for fla^+ with uvr^+ in transductions into *uvrC*, *fla* mutants confirm this clustering (Table 6). *flaA*, *B*, *C*, and *E* all show between 25 and 35% cotransduction with uvr^+, whereas *flaD* and *hag* give 64 and 67% cotransduction. Figure 6 summarizes the results of these crosses and compares them to previous results by Armstrong and Adler (3).

DISCUSSION

The use of merodiploid strains with flagellar mutations on the exogenote and endogenote

TABLE 2. *Rescue of hag-207 gene product by F elements*[a]

Merodiploid		Rescue of *hag-207*[b]
Endogenote	Exogenote	
flaA41 hag-207	*fla*$^+$ *hag-208*	Yes
flaA1004 hag-207	*fla*$^+$ *hag-208*	Yes
flaA1083 hag-207	*fla*$^+$ *hag-208*	Yes
flaB111 hag-207	*fla*$^+$ *hag-208*	Yes
flaB394 hag-207	*fla*$^+$ *hag-208*	Yes
flaB835 hag-207	*fla*$^+$ *hag-208*	Yes
flaC101 hag-207	*fla*$^+$ *hag-208*	Yes
flaC8012 hag-207	*fla*$^+$ *hag-208*	Yes
flaD867 hag-207	*fla*$^+$ *hag-208*	Yes
flaD886 hag-207	*fla*$^+$ *hag-208*	Yes
flaE234 hag-207	*fla*$^+$ *hag-208*	Yes
flaE1011 hag-207	*fla*$^+$ *hag-208*	Yes
flaF726 hag-207	*fla*$^+$ *hag-208*	No
flaF912 hag-207	*fla*$^+$ *hag-208*	No

[a] F1334 with a *hag-208* gene substitution for *hag-207*.

[b] Judged by the prevention of movement through motility agar containing anti-Hag 207.

TABLE 3. *Rescue of hag-207 gene product in merodiploids*

Endogenote[a]	Exogenote	*hag-207* product[b] (μg)
fla$^+$ *hag-208*	*flaA41 hag-207*	0.6
fla$^+$ *hag-208*	*flaA1004 hag-207*	0.6
fla$^+$ *hag-208*	*flaA1083 hag-207*	0.4
fla$^+$ *hag-208*	*flaB111 hag-207*	0.4
fla$^+$ *hag-208*	*flaB394 hag-207*	0.25
fla$^+$ *hag-208*	*flaB835 hag-207*	0.4
fla$^+$ *hag-208*	*flaC101 hag-207*	0.3
fla$^+$ *hag-208*	*flaC8012 hag-207*	0.4
fla$^+$ *hag-208*	*flaD867 hag-207*	0.5
fla$^+$ *hag-208*	*flaD886 hag-207*	0.5
fla$^+$ *hag-208*	*flaE234 hag-207*	0.4
fla$^+$ *hag-208*	*flaE1011 hag-207*	0.4
fla$^+$ *hag-208*	*flaF726 hag-207*	0.0
fla$^+$ *hag-208*	*flaF987 hag-207*	0.0

[a] The recipient is MS1032 and produces no *hag-207* product. *hag-207 fla*$^+$ haploid (MS1350) produces about 2 μg for 1 ml of cells at 2×10^8 cells/ml.

[b] Micrograms of protein estimated by complement fixation analysis on whole formalized cells at 2×10^8 cells/ml.

provided a reliable measure of complementation because the state of the exogenote in the *rec* strain could be accurately ascertained (17). Nevertheless, certain difficulties did arise with this method. (i) Gal$^+$ strains merodiploid in regions II and III became mucoid and synthesized very few flagella, which necessitated the use of *galU* recipients. The nature of this mucoid effect is unknown, and the effect has been observed by a number of laboratories (B. Low, J. A. Parkinson, *personal communication*). (ii) The *rec* marker had to be introduced into the recipients. (iii) F elements often degenerated with the deletion of the *uvrC* locus and the flagellar genes and thus had to be maintained in *rec* strains where they were stable.

TABLE 4. *Frequency of joint cotransduction of various alleles with his*$^+$

Donor genotype[a]	Recipient genotype	Recombinants			
		His$^+$ Fla$^+$/ total His$^+$		His$^+$ Fla$^+$ Uvr$^+$/total His$^+$	
		Ratio	%	Ratio	%
his$^+$ *fla*$^+$	*his hag-912*[b]	73/1,980	3.7	2/1,980	0.1
his$^+$ *fla*$^+$	*his flaB394*	66/1,540	4.3	3/1,540	0.2
his$^+$ *fla*$^+$	*his flaA1004*	66/1,155	5.7	0/1,855	0.0

[a] Donor strain is MS1380.

[b] The designation *flaF912* was changed to *hag-912* after the *F* locus was found to be in the *hag* gene. The designation of all other group *F* mutations were similarly changed by substitution of the *hag* prefix.

Mutations that failed to complement each other were placed in the same cistron, but there were two exceptions. (i) Mutations in the same cistron sometimes complemented each other, usually poorly; (ii) some mutations appeared to belong to more than one cistron. The observation of some complementation within a class of mutations in the same gene, called partial complementation, is common in the flagellar system (1, 2, 8, 9) and intra-allelic complementation in other systems is well documented (6, 16). Mutations that displayed partial complementation could still be placed in cistrons on the basis of their relationships with other mutations in the same group that did not exhibit partial complementation. The three strains that were found to carry mutations belonging in two cistrons could have resulted from a polarity effect. The following evidence supports this conclusion: (i) all three mutations were suppressible amber mutations, and (ii) all three fell into cistrons *flaB* and *flaC* which were found to be very closely linked and could be adjacent. Mutants such as these could be very helpful in revealing the organization of the *fla* genes into operons.

With due consideration for partial complementation and polar effects, a complementation map was assembled which indicated six cistrons in region III and four cistrons in region II. The region III cistrons were assigned the

TABLE 5. *Three-factor crosses*

Donor genotype[a]	Recipient genotype	Uvr⁺ Fla⁺ recombinants/Uvr⁺ recombinants				Order
		Transduction shown at left		Reciprocal transduction		
		Ratio	%	Ratio	%	
flaE234	*flaA1004*	16/480	3.3	12/480	2.5	—[b]
flaE234	*flaB394*	8/400	2.0	1/400	0.2	*flaE flaB uvrC*
flaE234	*flaC8012*	17/480	3.6	4/480	0.8	*flaE flaC uvrC*
flaE234	*flaD691*	28/240	11.6	4/240	1.6	*flaE flaD uvrC*
flaE234	*hag-912*	70/240	29.1	9/240	3.8	*flaE hag uvrC*
flaA1004	*flaB394*	28/480	5.8	18/480	3.8	—[b]
flaA1004	*flaC8012*	35/480	7.5	20/480	4.2	*flaA flaC uvrC*
flaA1004	*flaD691*	69/320	21.5	5/320	1.6	*flaA flaD uvrC*
flaA1004	*hag-912*	71/320	22.2	11/320	3.4	*flaA hag uvrC*
flaB394	*flaC8012*	1/480	0.2	1/480	0.2	—[b]
flaB394	*flaD691*	51/320	15.9	8/320	2.5	*flaB flaD uvrC*
flaB394	*hag-912*	51/320	15.9	11/320	3.4	*flaB hag uvrC*
flaC8012	*flaD691*	103/480	21.4	18/480	3.8	*flaC flaD uvrC*
flaC8012	*hag-912*	56/320	17.5	9/320	2.8	*flaC hag uvrC*
flaD691	*hag-912*	15/480	3.1	19/480	4.0	—[b]

[a] Donors are *uvr⁺ fla* derivatives of strain MS1350.
[b] Mutant loci too close to order.

TABLE 6. *Region III: cotransduction of fla⁺ with uvrC*

Donor genotype[a]	Recipient genotype	Fla⁺/Uvr⁺ [b]	
		Ratio	%
uvr⁺ fla⁺	*uvrC flaA1004*	147/440	33
uvr⁺ fla⁺	*uvrC flaB384*	119/440	27
uvr⁺ fla⁺	*uvrC flaC8012*	111/440	25
uvr⁺ fla⁺	*uvrC flaE234*	124/440	28
uvr⁺ fla⁺	*uvrC flaD691*	280/440	64
uvr⁺ fla⁺	*uvrC hag-912*	304/440	69

[a] Donor lysate was grown on strain MS1380.
[b] *uvr⁺* was selected marker.

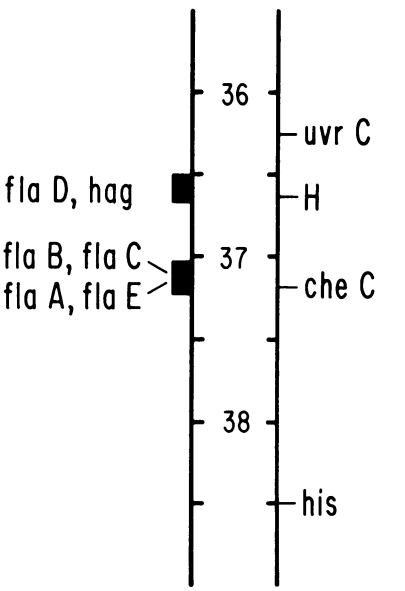

FIG. 6. *Clustering of flagellar genes in region III. Comparison of the location on the E. coli map of cistrons determined in this study (left column) with the location of flagellar genes mapped by Armstrong and Adler (3) (right column).*

letters *flaA, B, C, D, E,* and *hag,* and the region II cistrons *flaG, H, I,* and *mot.* These letters do not correspond to those used for the description of genes in *Salmonella.* However, having defined these cistrons in *E. coli,* it may be possible with interspecific mating to relate them to the corresponding cistrons in *Salmonella* (8, 9, 23). The possibility remains that some *fla*-associated cistrons were not detected. In fact, the χ phage procedure would not be effective in selecting chemotaxis or other groups of mutations that could have subtle effects on flagellar structure and activity.

Electron microscope observation of all of the region II and III mutants participating in complementation analysis and representatives from region I was performed. The results were consistent with the data obtained by complementation analysis. All members of complementation group *J* had flagella but were incapable of translational motion and were thus classified as *mot* mutants. All mutants in complementation group *E* produced filaments of very low wavelength (~0.14 μm) which were 1 to 2 μm long. This "polyhook" is attached directly to the basal assembly, and a filament composed of flagellin can be found distal to it. We have

characterized this defective structure (18) and call it a polyhook because it appears to result from an abnormal termination of the hook structure. Members of complementation group *F* were incapable of producing flagellin, as demonstrated by attempts to rescue the *hag* gene product in partial diploids. Furthermore, two strains with mutations in the *F* cistron produce a protein that cross-reacts with anti-flagellin antisera. This protein was detected in supernatant fluids obtained by disrupting MS912 and MS987, but not in any other *fla* mutant strains so far tested (Simon and Silverman, *unpublished results*). We are examining these strains, which carry amber mutations, for the production of abbreviated flagellin molecules. They may also be useful in studying the regulation of flagellin production. Mutants that accumulate internal flagellin pools have previously been described by Iino (8) in work with *Salmonella*.

Complementation analysis of region I mutants was not successful because the KLF26 episome could not be manipulated easily because of its infertility and instability. Efforts to generate smaller, fertile episomes useful in this region have failed so far and other approaches are being tested.

The cotransduction of *fla*$^+$ and *uvr*$^+$ with *his*$^+$ as the selected marker demonstrated unequivocally that several cistrons assigned to region III (*flaA*, *B*, and *hag*) were between *his* and *uvrC*. Three-point crosses established the relationship of the other cistrons of region III to *flaA*, *B*, and *hag*. It is evident that all six cistrons tentatively assigned to region III are in fact in region III. Furthermore, *flaE*, *A*, *B*, and *C* were found to be very close to each other and possibly adjacent, while *flaD* and *hag* are farther away and possibly adjacent to each other. Cotransduction of *fla*$^+$ with *uvr*$^+$ confirmed this clustering. The observation of amber mutants showing polar effects between *flaB* and *flaC* is consistent with the close association of these genes. These data were used to obtain a map of the flagellar genes in region III of the *E. coli* chromosome (Fig. 6). The *cheC* locus which was defined by Armstrong and Adler (3) appears to lie adjacent to the *flaB*, *C*, *A*, *E* cluster. These genes may form a regulatory unit. We are preparing to study the organization of region III genes into operons by using polar amber mutations and deletion mapping.

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