JOURNAL OF BACTERIOLOGY, Sept. 1977, p. 801-808

Vol. 131, No. 3
Printed in U.S.A.

Genetic Analysis of *Escherichia coli* K-12 Region I Flagellar Mutants

Y. KOMEDA,[1] M. SILVERMAN, AND M. SIMON*

Department of Biology, University of California, San Diego, La Jolla, California 92093

Received for publication 2 May 1977

Flagellar mutants in *Escherichia coli* region I were obtained by selection for resistance to the flagellotropic phage χ. F′ elements carrying this region of the *E. coli* genome were then constructed. Stable merodiploid strains with a flagellar defect on the exogenote and another on the endogenote were prepared. These merodiploids yielded information on the complementation behavior of mutations in this region. Region I was shown to include at least six cistrons, *flaV*, *flaK*, *flaL*, *flaM*, *flaS*, and *flaT*. Mu-induced and deletion *fla* mutants were also isolated. By using these mutant strains, the transcriptional order was shown to be *flaV-flaK-flaL-flaM-flaS-flaT*. The definition of region I *fla* genes and their transcriptional relationships were confirmed by genetic tests with hybrid λ phage carrying *fla* genes in this region.

The flagellar apparatus in bacteria is a model system for the study of the morphogenesis of subcellular organelles and for the study of energy conversion and sensory transduction. Genetic analysis has been a valuable tool for the elucidation of these processes.

Flagellar assembly has been shown to require about 16 genes, called *fla* or *hag*, in *Escherichia coli* K-12 (9, 15–17) and 15 genes in *Salmonella* (8, 18, 19). In *E. coli*, the *fla* genes were assigned to three regions of the genome (1). The region I *fla* genes were mapped near *pyrC*, those of region II were mapped between *aroD* and *uvrC*, and those of region III were mapped in the region between *uvrC* and *his*. So far, extensive studies have focused on region II and III *fla* genes (9, 14–17; see Fig. 2).

Genetic analysis of region I showed one transcriptional unit consisting of three genes, *flaK*, *flaL*, and *flaM* (M. Silverman, Ph.D. thesis, University of California, San Diego, La Jolla, Calif., 1972). Recently, hybrid plasmids containing region I *fla* genes were shown to program the synthesis of several proteins of the basal structure of the flagellar organelle, including the protein subunit of the flagellar hook (11). Since the region I *fla* genes code for the synthesis of components of the flagellar rotor, it is particularly important that a precise genetic study of this region be undertaken. Therefore, many additional flagellar mutants with defects in region I were isolated. These included missense, nonsense, deletion, and polar Mu phage-induced mutants. Complementation analysis was performed with merodiploid strains constructed with region I *fla* mutations on an F′ element (exogenote) and on the endogenote. Hybrid λ were also constructed that contained region I *fla* genes, and these were useful in defining and mapping the *fla* genes.

[1] On leave from: Laboratory of Genetics, Faculty of Science, University of Tokyo, Tokyo, Japan.

MATERIALS AND METHODS

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, 10 g; yeast extract (Difco), 5 g; and thymine, 0.1 g. 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 with a supplement of 0.1 g of thymine per liter.

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 bases, if required, were added to a final concentration of 100 mg per liter. Minimal agar plates were prepared by adding 1.5% agar to minimal medium. Minimal motility plates were prepared by substituting glycerol for glucose and adding 0.35% agar to minimal medium.

Bacterial strains. The *E. coli* K-12 strains are listed in Table 1 with their genotypes and derivations. The Fla mutants described here were derived from strains MS1350 and YK102. The preparation of strain MS1350 has been previously described (15). Strain YK102 was prepared from MS22, which is an *flaL* mutant of strain MS1350. Strain MS22 was made *flaL*$^+$ *pyrC46* by P1 cotransduction from strain 30SO-U6 (*fla*$^+$ *pyrC46*) and then *nalA* by selection

TABLE 1. *E. coli K-12 strains used*[a]

Strain	Relevant genotype	Construction/source
MS1350	F⁻ *his argE thyA galU thi strA uvrC mtl xyl*	This laboratory (15)
MS22 *recA*	MS1350 *flaL22 recA*	This laboratory
YK101	F⁻ *his argE thyA galU thi strA uvrC mtl xyl pyrC46*	Transductant of MS22 from 30SO-U6
YK102	YK101 *nalA*	Nalidixic acid resistant
YK103	YK102 *recA thy*⁺	Recombinant from conjugation of YK102 with JC5072
YK106	YK102 (λ *fla69*)	Lysogen of YK102
YK107	YK103/F'KLF23 (*galU*⁺)	F-ductant of YK103 with KLF23
YK108	YK102 (λ *fla6*)	Lysogen of YK102
YK110	YK102 Δ(*flaVKLMST*) *recA*	This study
X7014L	F⁻ *pyrC46 purB51 thi-1 str-121*	W. Epstein
YK1001	X7014L *thyA*	*thyA* of X7014L
YK1002	YK1001 Δ(*flaNBCOEAPQR*) *thy*⁺	Recombinant from conjugation of YK1001 with EJ780
YK1003	YK1002 *thyA*	*thyA* of YK1002
YK1004	YK1003 *recA*	*recA* of YK1003
YKF1005	YK1004/F'1005 (*pyrC*⁺ *purB*⁺ *flaVKLMST*⁺)	This study
KL99	Hfr *thi-1 relA lac-42*	This laboratory
EJ780	Hfr Δ(*gal attλ chlA bio uvrB*) *thi* Δ(*flaNBCOEAPQR*)	Y. Komeda (7)
30SO-U6	Hfr, *thi-1 pyrC46 relA1 lacZ43*	B. Bachmann (CGSC 5153)
JC5072	Hfr *thr leu recA67*	This laboratory (15)
PCO254	F⁻ *thi-1 tyrA2 his-68 trp-45 purB51 lacY1 malA1 mtl-2 xyl-7 gal-6 strA125 tonA21 tsx-70 supE44*	B. Bachmann (CGSC5038)
MS1500	F⁻ *his galU uvrC thi strA argE recA67*/KLF23	This laboratory (16)

[a] Δ, Deletion.

for nalidixic acid resistance on L agar containing 10 μg of nalidixic acid per ml. Since MS1350 was *galU*, it was highly permeable to the drug (6). Therefore, 10-μg/ml resistance was the highest resistance that could be isolated. Strain YK102 had a 100-μg/ml resistance if *galU*⁺ was introduced by transfer of KLF23 (*galU*⁺). The repository strain for F' elements was YK1004, constructed as follows. A ThyA mutant of X7014L (strain YK1001) was selected by trimethoprim selection (16). A deletion of *flaN* through *flaR* (region III; 15) was introduced into YK1001 from strain EJ780 by Hfr conjugation to obtain strain YK1002. Selection was for Thy⁺ Str^r recombinants. A *thyA* mutant, YK1003, was isolated from strain YK1002 by trimethoprim selection. Strain YK1003 was crossed with Hfr strain JC5072 to introduce the *recA67* mutation. A RecA clone was found among the Thy⁺ Str^r recombinants of the cross, and this strain, YK1004, was used as the repository strain for F' elements containing region I flagellar genes. Since this strain had selective markers, F' elements containing region I *fla* gene mutations could be transferred easily into and out of this strain. In addition, this strain was *recA*, so there would be no exchange between endogenote and exogenote *fla* loci.

Isolation of mutants. In addition to ethyl methane sulfonate-induced *fla* mutants already available (15), three kinds of flagellar mutants were isolated.

(i) **Spontaneous mutants.** Spontaneous mutants were selected for their resistance to the flagellotropic phage χ (12). Phage-resistant clone 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 and 0.1 ml of χ phage (10^9 plaque-forming units per ml). Survivors were streaked twice on L agar plates and then tested for motility.

(ii) **Mu-induced mutants.** Bacteriophage Mu-induced *fla* mutants were isolated from strain YK107. YK107 was a *recA* derivative of YK102 and was Gal⁺ because it harbored F' KLF23, which contained the *galU*⁺ gene. Mu phage infect only Gal⁺ bacteria (16). The mutagenesis with Mu phage was performed as described previously (16). Flagellar mutants were selected from Mu phage-infected cultures, and only Fla strains that were Mu phage lysogens were saved.

(iii) **Deletion mutants.** Selection of flagellar deletion mutants by λ eduction has been reported (9). Strains YK106 and YK108 were derivatives of YK102 lysogenic for λ*fla69* and λ*fla6*, respectively. The construction of these hybrid λ phage is described below. The hybrid lambda were presumably integrated into region I flagellar genes, because they had no λ attachment site but carried deoxyribonucleic acid homologous to region I. The prophages carried the cI857 mutation and were, therefore, heat inducible (40°C). Clones that survived at 40°C were selected. Some of these were expected to have dele-

tions that extended into the flagellar genes. However, nonmotile clones appeared at a frequency of less than 10^{-3} of the survivors. So, we used chi phage for the selection of *fla* deletion mutants. An overnight culture of strain YK106 or strain YK108 was incubated at 42°C for 15 min, plated on L agar plates with chi phage, using soft agar as in (i), and incubated at 40°C overnight. The clones harboring resistance to chi phage infection appeared at almost the same frequency as those of control culture without heat-pulse and incubation at 40°C. The stable heat-resistant *fla* mutants were chosen and checked for their mutation sites.

F′ contruction. F′ elements were generated by the method of Low (10) by conjugating Hfr KL99, which donated *pyrC*$^+$ as the proximal marker, with strain MS22 *recA*. The mixed culture of strains KL99 and MS22 *recA* was streaked on motility agar containing streptomycin (200 μg/ml) for counterselection of the donor. Swarm formers were selected (>100 were saved), and 25 clones had the MS22 *recA* genotype, also showing sensitivity to male-specific phage MS2. These were used as donors to transfer *pyrC*$^+$ *purB*$^+$ markers to a repository strain, YK1004. One of the clones (MS22 *recA* motile clone 13) transferred the markers at high frequency, and a resultant F-ductant in strain YK1004 was named strain YKF1005. The F′ element was designated F′1005. This F′ element did not carry the *fabA* or the *galU* genes.

Complementation analysis with merodiploid strains. Complementation analysis with F′1005 required the construction of merodiploid strains carrying different flagellar defects on the exogenote and endogenote. This necessitated the introduction of *recA* into Fla mutants and the transfer of the flagellar mutations to the episome. The procedures for introduction of *fla* mutations into the F′ element were described previously (15). Mutations in region I *fla* genes were introduced into the F′1005 element. Strain YK110 was a region I *fla* deletion mutant that had no intact *fla* gene in region I. It carried a *recA* mutation and harbored the F′1005 element. Region I *fla* mutants of the F′ were selected in this strain, and their presence was confirmed by genetic tests with the λ*fla6*91 phage. These region I *fla* mutants served as donor strains in F′ mapping and complementation analysis.

Mating was accomplished by replicating the recipient strains on an L broth agar lawn of donor strain (YKF1005, carrying flagellar mutations on the F′ element) by using a brass block replicator with 48 needles. After 10 h of incubation of the mating bacteria at 37°C, cells were transferred to a minimal agar plate containing 10 μg nalidixic acid per ml for counterselection of the donor by nalidixic acid and of the recipient by uracil deprivation. After incubation for 36 h at 37°C, the replicator was used to transfer exconjugant bacteria to a minimal-motility agar plate, which further selected for PyrC$^+$ exconjugant bacteria. Motility was compared after 48 h of incubation. Motility indicated complementation of *fla* defects in Rec cells. In Rec$^+$ cells, motility was often the result of restoration of a nondefective genotype by recombination.

Isolation of hybrid lambda transducing phages carrying region I flagellar genes. Initially, a λ hybrid with some of the region I *fla* genes was isolated by using λ*gt*λ*c* deoxyribonucleic acid as the vehicle and fragments of deoxyribonucleic acid obtained by *Eco*RI endonuclease digestion of a ColE1-*E. coli* deoxyribonucleic acid hybrid (pLC 36-11) carrying region I flagellar genes (see Clarke and Carbon, 2). The details of this construction were identical to those used to construct other λ*fla* hybrids (14). The hybrid lambda, λ*fla6*, was integrated into the genome of strain YK102. Hybrid lambda transducing phage were derived from the lysogen of strain YK102 by subsequent heat induction. The precise experimental procedures are the subject of another report (manuscript in preparation).

Deletion mutants of the hybrid were isolated by the pyrophosphate shock method (13). The construction of these hybrid λ is outlined in Fig. 1.

RESULTS

Isolation and complementation analysis of region I *fla* mutants. Fla and Mot mutants were obtained by χ phage selection. Among these, region I Fla mutants were identified by screening with F′1005, which complemented only region I mutants. We isolated 26 and 20 region I *fla* mutants from strains YK102 and YK103, respectively. F′ elements (F′1005) carrying various flagellar defects in region I were collected by means of recombination with

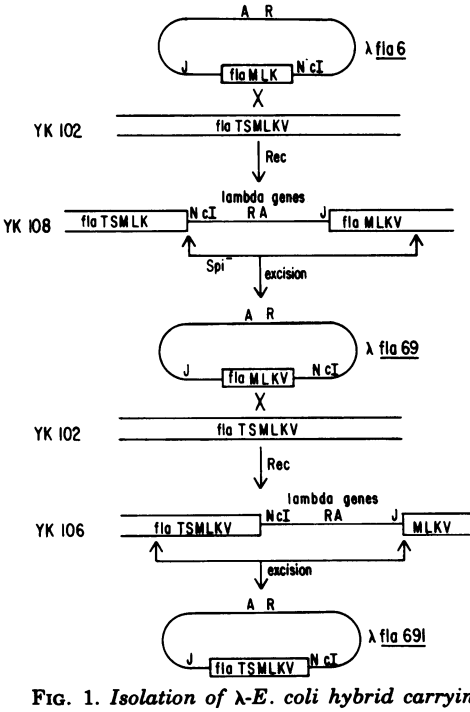

FIG. 1. *Isolation of λ-E. coli hybrid carrying region I flagellar genes.*

Rec$^+$ merodiploid strains or by isolation of *fla* mutants on the F′ element from strain YK110. To study complementation between different flagellar mutations, merodiploid strains were constructed with different flagellar defects on the exogenote and endogenote. For this purpose, the recipient strains were made Rec (*recA*). Complementation could be determined by observing swarm formation from the Rec merodiploid strains on motility agar. The complementation behavior of flagellar mutants obtained in this manner indicated that there were at least six cistrons in region I (Table 2). These complementation groups were designated *flaV*, *flaK*, *flaL*, *flaM*, *flaS*, and *flaT*.

As described in Materials and Methods, nonreverting *fla* mutants were isolated from strains YK108 and YK106. These mutations were examined by using F′ elements carrying various *fla* mutations. At first, Rec$^+$ strains were used, and the results are shown in Table 3. All of the *fla* mutations behaved in genetic crosses as multisite mutations. From the isolation procedure, it is conceivable that they are deletion mutants. Merodiploid analysis was repeated with *recA* derivatives of these *fla* mutants. The results are also shown in Table 3. None of the deletion mutants showed polarity effects on gene expression. One of the deletion mutants, strain YK2258, did not have any region I *fla* genes intact. This strain was also shown to have no region I *fla* genes intact by P1-mediated transduction. This is the parental strain of strain YK110. YK2254 behaved as if it carried a deletion that removed most of *flaL* and part of *flaM*, since in the Rec$^+$ cross it formed recombinants with the *flaM* point mutant exogenotes. The order that resulted was: (*flaV*, *flaK*), *flaL*, *flaM*, (*flaS*, *flaT*). The order of the genes in parentheses was not apparent from this analysis.

Isolation and complementation analysis of bacteriophage Mu-induced Fla mutants. Mu-induced flagellar mutants were isolated from strain YK107. Seventy-five Mu-lysogenic strains with defective flagellar functions were examined by cross-streaking with λ*fla*691, and lesions in 15 were assigned to region I. These Mu-induced flagellar mutants were analyzed by using F′ elements carrying various flagellar defects in region I. Examination of the 15 mutant strains was carried out, and Table 4 shows the results of the complementation tests. Three of the 15 mutants did not show polarity of gene expression. These may represent point mutants unexpectedly isolated by this procedure. The other 12 mutants showed polarity effects; that is, the Mu phage insertion affected the expression of more than one gene. The results clearly indicate the existence of an operon containing all the region I *fla* genes. The gene order is *flaV*, *flaK*, *flaL*, *flaM*, *flaS*, *flaT*. This is in agreement with the order suggested by the examination of deletion mutants in region I *fla* genes.

Genetic analysis with hybrid λ*fla*. The use of transducing phage for genetic analysis has been well documented (14), and the occurrence of trails of immotile colonies on motility agar (abortive transductants) is an indication of complementation of *fla* defects. The occurrence of motile swarms results from complementation by stable integration of the transducing phage or by recombination to a nondefective genotype. Hybrid λ*fla*6 could make swarms and trails in transductional crosses with strains carrying *flaV*, *flaK*, *flaL*, and *flaM* mutants nonlysogenic for λ. When lysogenic mutants were used, λ*fla*6 could not make trails. This suggested that *fla* gene expression on λ*fla*6 required a λ promotor and that this hybrid λ*fla*6 contained an incomplete region I operon. Therefore, λ hybrids were sought that contained the entire region I operon. The isolation of these hybrids was described in Materials and Methods and in Fig. 1. λ*fla*69 could make trails on some region I Fla mutants lysogenic for λ. Hybrid λ*fla*691 from strain YK106 (strain YK102 lysogenic for λ*fla*69) carried all the region I genes. As can be seen from Fig. 1, two eduction events were required to obtain the entire operon. Deletion mutants from λ*fla*69 and λ*fla*691 were isolated by the pyrophosphate shock method and were characterized genetically by using various region I *fla* mutants lysogenic for λ in transductional crosses. Table 5 shows the results of the complementation analysis. The gene order that resulted was: *flaV*, *flaK*, *flaL*, *flaM*, (*flaS*, *flaT*). This order agrees with that obtained by the analysis of Mu-induced *fla* mutants.

The derivatives of strain MS1350 isolated by Silverman and Simon (15), could not be mapped by F′ element 1005, because the strains did not have the *pyrC* marker for selection. Therefore, these were mapped by transductional crosses on motility agar at 30°C with λ*fla* derivatives isolated and characterized as shown above. Table 6 lists the results. These mutants could clearly be assigned to the *fla* cistrons defined in this study, but specific assignment to *flaS* or *flaT* was not possible. Additional transductional crosses with the Fla mutants derived from strain YK102 were performed. The same results as shown in Table 6 were obtained, and they were in agreement with the mapping and complementatior analysis obtained with mero-

TABLE 2. *Summary of complementation behavior between flagellar mutants*[a]

	Donor										
Recipient	2456	2003	2453	2015	2451	2038	2470	2001	2065	2002	Cistron
2007	−	+	+	+	+	+	+	+	+	+	*flaV*
2022	−	+	+	+	+	+	+	+	+	+	
2023	−	+	+	+	+	+	+	+	+	+	
2054	−	+	+	+	+	+	+	+	+	+	
2114	−	+	+	+	+	+	+	+	+	+	
2115	−	+	+	+	+	+	+	+	+	+	
2121	−	+	+	+	+	+	+	+	+	+	
2003	+	−	−	+	+	+	+	+	+	+	*flaK*
2008	+	−	−	+	+	+	+	+	+	+	
2033	+	−	−	+	+	+	+	+	+	+	
2034	−	−	−	+	+	+	+	+	+	+	
2037	+	−	−	+	+	+	+	+	+	+	
2055	+	−	−	+	+	+	+	+	+	+	
2058	+	−	−	+	+	+	+	+	+	+	
2101	+	−	−	+	+	+	+	+	+	+	
2122	+	−	−	+	+	+	+	+	+	+	
2125	+	−	−	+	+	+	+	+	+	+	
2132	+	−	−	+	+	+	+	+	+	+	
2139	+	−	−	+	+	+	+	+	+	+	
2015	+	+	+	−	−	+	+	+	+	+	*flaL*
2040	+	+	+	−	−	+	+	+	+	+	
2045	+	+	+	−	−	+	+	+	+	+	
2046	+	+	+	−	−	+	+	+	+	+	
2063	+	+	+	−	−	+	+	+	+	+	
2105	+	+	+	−	−	+	+	+	+	+	
2106	+	+	+	−	−	+	+	+	+	+	
2107	+	+	+	−	−	+	+	+	+	+	
2124	+	+	+	−	−	+	+	+	+	+	
2138	+	+	+	−	−	+	+	+	+	+	
2017	+	+	+	+	+	−	−	+	+	+	*flaM*
2038	+	+	+	+	+	−	−	+	+	+	
2047	+	+	+	+	+	−	−	+	+	+	
2068	+	+	+	+	+	−	−	+	+	+	
2104	+	+	+	+	+	−	−	+	+	+	
2112	+	+	+	+	+	−	−	+	+	+	
2116	+	+	+	+	+	−	−	+	+	+	
2117	+	+	+	+	+	−	−	+	+	+	
2134	+	+	+	+	+	−	−	+	+	+	
2001	+	+	+	+	+	+	+	−	−	+	*flaS*
2073	+	+	+	+	+	+	+	−	−	+	
2078	+	+	+	+	+	+	+	−	−	+	
2002	+	+	+	+	+	+	+	+	+	−	*flaT*
2077	+	+	+	+	+	+	+	+	+	−	
2119	+	+	+	+	+	+	+	+	+	−	
2111	−	−	−	−	−	−	−	−	−	−	
2131	−	−	−	−	−	−	−	−	−	−	

[a] Symbols: +, complementation; −, no complementation.

diploid strains (Table 2).

Mapping of region I fla genes on *E. coli* chromosome. Two-point transductional crosses were performed using P1*kc*-mediated transduction. *PyrC*$^+$ was cotransduced 61% with region I *fla* mutations. According to Wu's function (20), the genes are located 0.3 min from *pyrC* on the *E. coli* map. However, we could not detect co-

TABLE 3. *Characterization of deletion flagellar mutants with F' fla strains*[a]

Recipient	*recA*	Parent	F' donor									
			2456 (*flaV*)	2003 (*flaK*)	2453 (*flaK*)	2015 (*flaL*)	2451 (*flaL*)	2038 (*flaM*)	2470 (*flaM*)	2001 (*flaS*)	2465 (*flaS*)	2002 (*flaT*)
2250	+	YK108	+	+	+	+	+	−	−	+	+	+
2253	+	YK108	−	−	−	−	−	−	−	+	+	+
2254	+	YK108	+	+	+	−	−	+	+	+	+	+
2255	+	YK108	+	+	+	−	−	+	+	+	+	+
2257	+	YK106	+	+	+	−	−	+	+	+	+	+
2258	+	YK106	−	−	−	−	−	−	−	−	−	−
2250	−	YK108	+	+	+	+	+	−	−	+	+	+
2253	−	YK108	−	−	−	−	−	−	−	+	+	+
2254	−	YK108	+	+	+	−	−	−	−	+	+	+
2255	−	YK108	+	+	+	−	−	+	+	+	+	+
2257	−	YK106	+	+	+	−	−	+	+	+	+	+
2258	−	YK106	−	−	−	−	−	−	−	−	−	−

[a] Symbols are the same as in Table 2.

TABLE 4. *Complementation analysis of Mu-induced flagellar mutants*[a]

Recipient	F' donor					
	2456 (*flaV*)	2453 (*flaK*)	2451 (*flaL*)	2470 (*flaM*)	2465 (*flaS*)	2002 (*flaT*)
2506	−	−	−	−	−	−
2507	−	−	−	−	−	−
2514	−	−	−	−	−	−
2516	−	−	−	−	−	−
2502	+	−	−	−	−	−
2503	+	+	−	−	−	−
2505	+	+	−	−	−	−
2515	+	+	−	−	−	−
2509	+	+	+	−	−	−
2504	+	+	+	+	−	−
2508	+	+	+	+	−	−
2501	+	+	+	+	+	−
2510	+	−	+	+	+	+
2512	+	+	−	+	+	+
2511	+	+	+	−	+	+

[a] Symbols are the same as in Table 2.

TABLE 5. *Characterization of λ-E. coli hybrids*[a]

Fla tester strain	Lambda						
	691	69Δ1	691Δ16	691Δ17	69	691Δ1	691Δ11
2007 (*flaV*)	+	+	+	+	+	−	−
2003 (*flaK*)	+	−	+	+	+	−	−
2015 (*flaL*)	+	−	−	+	+	−	−
2017 (*flaM*)	+	−	−	−	+	+	+
2001 (*flaS*)	+	−	−	−	−	+	+
2002 (*flaT*)	+	−	−	−	−	+	+

[a] Symbols are the same as in Table 2.

transduction between *purB* and region I and could not determine on which side of *pyrC* region I *fla* genes reside (Fig. 2).

DISCUSSION

We identified six cistrons in flagellar region I by the use of merodiploid strains. An F' element carrying the genes in region I was constructed. Many point mutations were introduced on the F' elements. Several were introduced by recombination with chromosomal *fla* mutations. The use of merodiploid strains with specific flagellar mutations on the exogenote and endogenote allowed the definition of com-

TABLE 6. *Complementation analysis of ethyl methane sulfonate-induced flagellar mutants derived from strain MS1350 with λ-E. coli hybrids*[a]

Fla mutant	Phage							
	λ69	λ691	λ69Δ1	λ691Δ16	λ691Δ17	λ691Δ11	λ691Δ1	Cistron
986	−	+	−	−	−	−	−	*flaVKLMST*
393	+	+	−	+	+	−	−	*flaK*
817	+	+	−	+	+	−	−	
232	+	+	−	+	+	−	−	
22	+	+	−	−	+	R[b]	−	*flaL*
32	+	+	−	−	+	R	−	
364	+	+	−	−	+	−	−	
633	+	+	−	−	+	−	−	
636	+	+	−	−	+	−	−	
676	+	+	−	−	+	−	−	
721	+	+	−	−	+	−	−	
762	+	+	−	−	+	−	−	
787	+	+	−	−	+	−	−	
809	+	+	−	−	+	−	−	
861	+	+	−	−	+	−	−	
874	+	+	−	−	+	−	−	
879	+	+	−	−	+	−	−	
911	+	+	−	−	+	−	−	
1056	+	+	−	−	+	−	−	
1102	+	+	−	−	+	−	−	
7610	+	+	−	−	+	−	−	
7711	+	+	−	−	+	−	−	
9510	+	+	−	−	+	−	−	
846	+	+	−	−	+	R	−	
1031	+	+	−	−	+	R	−	
885	+	+	+	+	+	−	−	*flaV*
971	+	+	+	+	+	−	−	
993	+	+	+	+	+	−	−	
1024	+	+	+	+	+	−	−	
9410	+	+	+	+	+	−	−	
656	+	+	−	−	−	+	+	*flaM*
755	+	+	−	−	−	+	+	
768	+	+	−	−	−	+	+	
952	+	+	−	−	−	+	+	
979	+	+	−	−	−	+	+	
1095	+	+	−	−	−	+	+	
8611	+	+	−	−	−	+	+	
9912	+	+	−	−	−	+	+	
7512	−	+	−	−	−	+	+	*flaS* or T
7910	−	+	−	−	−	+	+	
51	−	+	−	−	−	+	+	

[a] Symbols are the same as in Table 2.
[b] R, Recombination (swarm formation).

plementation groups. This definition was confirmed by transductional crosses with a variety of hybrid λ phage containing region I *fla* genes.

As a result of the analysis of Mu-induced mutants, it was shown that the *fla* genes in region I were cotranscribed, with the order of transcription being *flaV-flaK-flaL-flaM-flaS-flaT*.

The genes in region I code for proteins of the hook-basal body structure (11; unpublished results). Previous work has shown that the synthesis of the hook subunit protein responded to the presence of the *flaI* gene product. *flaI* presumably has a role in regulating the expression of the genes in the region I operon (4, 5). *flaI* acts as a positive controlling element. It will be

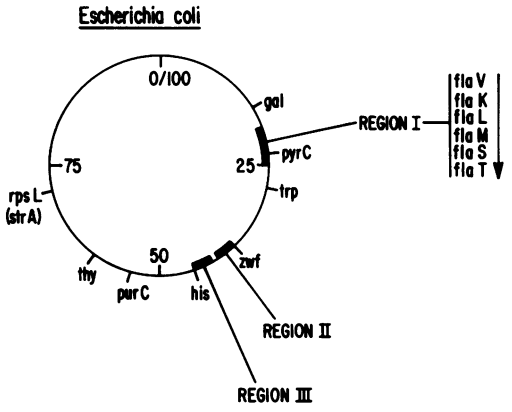

FIG. 2. *Chromosomal map of E. coli flagellar genes. The relative orientation of fla with pyrC is unknown.*

of interest to determine the mechanisms involved in the interaction of these gene products. With the lambda phages carrying these genes, it should be possible to make specific gene product assignments by using the hybrid λ to program protein synthesis in ultraviolet-irradiated cells (14). The identification of the gene products that assemble to form the flagellar rotor should make it possible to understand the structure of the rotor and its relationship to the cell membrane (3, 4, 5).

The mechanism of the coupling of membrane energy to flagellar rotation will surely become clear when the components of the flagellar apparatus are more clearly identified. Isolation of the hook-basal complex has shown that there are at least 11 polypeptides, including flagellin, involved in the formation of the structure. Even if all of the gene products formed in region I were part of the structure, they would not account for all of its components. Presumably, the rest of the components are controlled by genes that map in region III or, perhaps, by the *flaG* or *flaH* genes, whose products have not yet been identified.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation and from the National Institutes of Health.

LITERATURE CITED

1. **Armstrong, J. B., and J. Adler.** 1967. Genetics of motility in *Escherichia coli:* complementation of paralyzed mutants. Genetics 56:363–373.
2. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic Col El hybrid plasmids representative of the entire *Escherichia coli* genome. Cell 9:91–99.
3. **DePamphilis, M. L., and J. Adler.** 1971. Purification of intact flagella from *Escherichia coli* and *Bacillus subtilis*. J. Bacteriol. 105:376–383.
4. **Hilmen, M., M. Silverman, and M. Simon.** 1974. The regulation of flagellar formation and function. J. Supramol. Struct. 2:360–371.
5. **Hilmen, M., and M. Simon.** 1976. Motility and the structure of bacterial flagella, p. 35–45. R. Goddson, T. Pollard, and J. Rosebaum (ed.), Cold Spring Harbor Conference on Cell Proliferation, vol. 3, Motility. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Komeda, Y., T. Icho, and T. Iino.** 1977. The effect of *galU* mutation on flagellar formation in *Escherichia coli*. J. Bacteriol. 129:908–915.
7. **Komeda, Y., K. Shimada, and T. Iino.** 1977. Isolation of specialized lambda transducing phages for flagellar genes (*fla*) of *Escherichia coli* K-12. J. Virol. 22:654–661.
8. **Komeda, Y., H. Suzuki, J. Ishidsu, and T. Iino.** 1975. The role of cAMP in flagellation of *Salmonella typhimurium*. Mol. Gen. Genet. 142:289–298.
9. **Kondoh, H., and H. Ozeki.** 1976. Deletion and amber mutants of *fla* loci in *Escherichia coli* K-12. Genetics 84:403–421.
10. **Low, B.** 1968. Formation of merodiploids in matings with a class of *rec*⁻ recipient strains of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. 60:160–167.
11. **Matsumura, P., M. Silverman, and M. Simon.** 1977. Cloning and expression of the flagellar hook gene on hybrid-plasmids in mini-cells. Nature (London) 265:758–760.
12. **Meynell, E. W.** 1961. A phage, $\phi\chi$, which attacks motile bacteria. J. Gen. Microbiol. 25:253–290.
13. **Parkinson, J. S., and R. J. Huskey.** 1971. Deletion mutants of bacteriophage lambda. J. Mol. Biol. 56:369–386.
14. **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.A. 73:3126–3130.
15. **Silverman, M., and M. Simon.** 1973. Genetic analysis of flagellar mutants in *Escherichia coli*. J. Bacteriol. 113:105–113.
16. **Silverman, M., and M. Simon.** 1973. Genetic analysis of bacteriophage Mu-induced flagellar mutants in *Escherichia coli*. J. Bacteriol. 116:114–122.
17. **Silverman, M., and M. Simon.** 1974. Positioning of flagellar genes in *Escherichia coli* by deletion analysis. J. Bacteriol. 117:73–79.
18. **Vary, P. S., and B. A. D. Stocker.** 1973. Nonsense motility mutants in *Salmonella typhimurium*. Genetics 73:229–245.
19. **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. 70:59–75.
20. **Wu, T. T.** 1966. A model for three point analysis of random general transductions. Genetics 54:405–410.