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Genetic Analysis of Bacteriophage Mu-Induced Flagellar Mutants in *Escherichia coli*

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In previous work, at least 10 discrete cistrons involved in the synthesis of flagella in *Escherichia coli* were described. Six cistrons were located between *his* and *uvrC* on the genetic map. These were referred to as *hag*, *flaA*, *flaB*, *flaC*, *flaD*, and *flaE*. Four cistrons referred to as *mot*, *flaG*, *flaH*, and *flaI* were located between *uvrC* and *aroD*. In order to determine whether these genes are organized into transcriptional units, a series of Mu phage-induced flagellar mutants was studied. The mutant strains behaved as if they were carrying strong polar mutations. Of 228 independent Mu-induced mutants, 114 with mutations in the *his-aroD* region of the genetic map were tested by preparing partial diploid strains with episomes carrying a variety of previously defined mutations. The pattern of complementation that emerged indicated that cistrons *flaB*, *flaC*, and *flaE* form a transcriptional unit. Cistron *flaO*, defined in the course of this study, is also a member of this transcriptional unit. The order of transcription is *B-C-O-E*. *flaA* was found to be complex, and it included four cistrons, *flaA*, *flaP*, *flaQ*, and *flaR*, with the transcriptional order *A-P-Q-R*. Cistrons *flaG* and *flaH* are cotranscribed with the transcriptional order *G-H*. The remaining genes, *flaD*, *flaI*, *hag*, and *mot* do not belong to multicistronic transcriptional units. Complementation analysis suggested that the *cheC* locus is the same as cistron *flaA*.

Although a great deal of effort has been expended defining genes necessary for flagellar synthesis and function in both *Escherichia coli* (2, 14) and *Salmonella* (8, 10, 18), little is known about the organization of these genes into transcriptional units. Analysis of the complementation behavior of amber mutants indicated that some flagellar genes might be cotranscribed (14). Critical examination of the organization of cistrons into transcriptional units would require the analysis of the influence of a mutation in one cistron on the expression of a nearby cistron. Such polar effects are difficult to demonstrate directly since very few of the products of the flagellar genes can be measured.

It is known that bacteriophage Mu-induced mutations are caused by the integration of the Mu prophage in the *E. coli* chromosome (15), and that the prophage can integrate into many different sites in a single gene (4-6). Furthermore, the integration of Mu into a gene has as one consequence the prevention of the expression of other genes in the same operon which are transcribed later (9, 16), and the Mu-induced mutation is, therefore, absolutely polar. Genes for ribosomal proteins have been shown to be transcribed as a unit by using Mu-induced mutations (12). With Mu-induced mutations as a source of polar mutations, a critical test of the organization of flagellin genes into transcriptional units would be possible.

Our analysis of the complementation behavior of Mu-induced flagellar mutations with previously defined point mutations in partial diploid strains of *E. coli* is the subject of this report. It has disclosed cotranscriptional relationships between cistrons which previously had not been suspected of belonging in the same operon. Three multicistronic units are now evident: *flaA-P-Q-R*, *flaB-C-O-E*, and *flaG-H*. Cistrons *flaD*, *flaI*, *hag*, and *mot* do not belong to multicistronic transcriptional units. The *cheC* mutation previously described by Adler and co-workers (1-3) is part of the *flaA* cistron. Cistron *flaO* was defined during the course of this work.

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ogy, 11 May 1973, in Miami, Florida.)

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; glucose, 2 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.

Bacteria. The *E. coli* K-12 strain, MS1350, with which most of our earlier flagella work was done, had to be changed in several ways to meet the requirements of this study. In order to perform complementation analysis, it was necessary to use Rec$^-$ strains, and MS1350 was made Rec$^-$ as previously described (14). Bacteriophage Mu would not infect strain MS1350 which is GalU$^-$, so the Rec$^-$ derivative strain MS1350 was made GalU$^+$ by transferring and F *trp*$^+$ *galU*$^+$ episome from strain KLF23 into this strain which would then support the growth and lysogenization of Mu. This derivative of MS1350 was designated MS1500 and has the genotype *his*, *galU*, *uvrC*, *sup*$^+$, *thi*, λ^-, *str*, *argE*, *hag207*, *recA67* (chromosomal), and *galU*$^+$ (episomal). Strain MS1500 is Fla$^+$ and is sensitive to the flagellotropic phage χ.

Three nonchemotactic strains were used in this study. One, contributed by J. Adler and derived from strain AW405, has a lesion in the chemotaxis locus which is designated *cheC497*. The other two were isolated by J. S. Parkinson (Univ. of Utah, manuscript in preparation) and derived from a *nalA*, *galE*, *sup*$^+$ derivative of strain AW405. These latter two were designated e20k1 and e21o2. Many point mutations in flagellar genes located on F factors were used in the complementation analysis described in this paper. The mutations, episomes, and strains harboring these factors were described in an earlier paper (14).

Isolation of mutants. Flagellar mutants were selected for their resistance to the flagellotropic phage χ (11) after Mu phage mutagenesis. The details of the χ selection were identical to those reported earlier (14). The mutagenesis with Mu phage was as performed by Borum and Abelson (4). In order to insure that each *fla* mutation was independent, only one flagellar mutant from each culture infected with Mu was retained. The Mu phage was a gift of J. Abelson. To test the efficacy of Mu phage as a mutagen, a comparison of the level of Mu-induced mutation to χ resistance with the level of spontaneous mutation to χ resistance was made. In a nonmutagenized population of cells, one χ-resistant mutant could be found in approximately 2×10^4 cells. In an Mu-infected population, one χ-resistant mutant could be found in approximately 4×10^2 cells or an enhancement of about 50-fold in the level of resistance by Mu phage infection. Therefore, most of the χ-resistant mutants would probably result from the action of Mu phage infection.

Mutant strains were given allele numbers and strain designations. The strain designation was derived from the allele number by adding the prefix MS. The 240 Mu-induced mutant strains isolated were given strain numbers MS1501 to MS1740. These mutant strains were tested for release of Mu phage by touching a soft agar overlay containing a Mu-sensitive strain, W3110, with a sterile toothpick carrying an inoculum of the Mu-induced flagellar mutant. A halo of lysis adjacent to the site of inoculation of the suspected lysogen indicated Mu release. Of the mutant strains, 239 demonstrated Mu release. To eliminate the possibility that the lysis of the indicator bacteria resulted from the release of some other lytic phage, the mutant strains were tested as before except on an Mu-resistant indicator strain WB-25 (a gift of J. Abelson). All 239 of the strains that showed lysis of strain W3110 failed to lyse Mu-resistant indicator strain WB-25, therefore the Mu-infected strains are probably Mu phage lysogens. To test the possibility that these strains harbor χ phage in some nonlytic state, the mutant strains were inoculated as before on a lawn of Fla$^+$ Mu lysogenic bacteria (Mu lysogenic MS1500) which were spread in a soft agar overlay on a motility agar plate. Of the 239 strains tested, one was found which released χ phage. The remaining 238 Mu lysogenic flagellar mutants were checked for reversion to Fla$^+$ and five were found to revert. These five were discarded because Mu-induced lesions have not been found to revert (6). Five strains grew very poorly on minimal medium and were not saved. The remainder (228) were saved for genetic analysis.

Mapping with F elements. Various F elements were used to locate the flagellar mutation on the *E. coli* chromosome. In this study, we were primarily concerned with the flagellar mutations in the *his-aroD* region. Flagellar mutant strains which could be converted to an Fla$^+$ phenotype by the transfer of the episome F1334 or F1338 were classified as region III mutants, that is, those strains which harbor defects in flagellar genes located between *his* and *uvrC*. Flagellar mutants which could be made Fla$^+$ by transfer of F1338 alone were classified as region II mutants, that is, those strains which harbor defects in flagellar genes between *uvrC* and *aroD*. F1334 carries these genes relevant to this study: *his*$^+$, *flq*$^+$ (region III), and *uvrC*$^+$. F1338 carries these genes: *his*$^+$, *fla*$^+$ (region III), *uvrC*$^+$, *fla*$^+$ (region II), and *zwf*$^+$. A more detailed description of these episomes and this method was presented earlier (14).

Complementation analysis. The construction of merodiploid strains carrying different flagellar defects on the exogenote and the endogenote is necessary for an analysis of the ability of pairs of mutations to complement each other to give a functional phenotype. In this study, F elements derived from either

F1334 or F1338 were transferred from a Rec$^-$ repository strain, JC1553, into the mutant strains to construct the merodiploid strains. The donor strains (see reference 14) donate an F element carrying these relevant genes: *his*$^+$ and either one of a series of region III flagellar defects on the episome (usually F1334) or one of a series of region II flagellar defects on the episome (usually F1338). Region III includes flagellar genes between *his* and *uvrC*, whereas region II includes flagellar genes between *uvrC* and *aroD* on the genetic map. The mating between donor and recipient (Mu-induced flagellar mutant) in this study was done on L agar plates. The donors were grown on a master plate overnight. An overnight culture of recipient in L broth (0.2 ml) was spread and dried on an L agar plate. The donor master was then replica-plated with a sterile velveteen pad onto the recipient plate. The mating plate was incubated for 6 h at 37 C, after which time the mating bacteria on the surface of this plate were replica-plated onto a minimal agar plate which selected for His$^+$ exconjugants of the recipient strain. The donor strain was counter-selected by exclusion of several amino acids from the medium which it required but which the recipient did not. After approximately 36 h the merodiploid bacteria harboring the F *his*$^+$ episomes appeared as patches of growth in the same position as the donor strain on the mating plate and donor master. Bacteria ($\sim 10^7$) from these patches were transferred to minimal motility plates where the ability of the merodiploid strain to swim through soft agar was tested. The minimal motility agar also would not support the growth of donor or recipient strain but only the His$^+$ merodiploid strain. Since the recipient strains are males (harbor F *galU*$^+$), it was thought necessary to cure the strains of this F element. These cured GalU$^-$ bacteria could be selected by infecting a soft agar overlay containing the GalU$^+$ bacteria with a suspension of P1*vir* phage (a gift of J. Abelson). A zone of lysis appeared where a drop of the phage suspension (titer $\cong 10^9$) was placed. Many of the P1*vir*-resistant clones were found to be Gal$^-$ since P1*vir* infects only GalU$^+$ bacteria (7). These bacteria did not harbor the F *galU*$^+$ any more and acted as females in matings with donor strains. However, the transfer of F elements into the male recipients (F *galU*$^+$) worked well without the curing of the F *galU*$^+$ episome presumably because the donor mated into F$^+$ phenocopy cells. Therefore, many matings were done without first selecting female recipients. The His$^+$ exconjugants selected in this manner apparently lost the F *galU*$^+$ episome because these strains were now GalU$^-$.

In this study the capacity for movement of merodiploid strains with defined point mutations on the exogenote and Mu-induced flagellar mutations on the endogenote were examined. Capability for movement indicated that complementation (+) had taken place and that the exogenote point mutation and the endogenote Mu-induced mutation were in different cistrons. If the merodiploid strain was incapable of movement, no complementation had taken place and the Mu insertion was assumed to have prevented the expression of a cistron identical to the defective cistron on the exogenote. If Mu insertion takes place in a gene which is a member of a multicistronic transcriptional unit, the expression of other genes transcribed after the gene in which the Mu insertion occurred will not be expressed, and the Mu insertion will have affected the expression of several genes. Therefore, Mu-induced mutations which by complementation analysis appear to belong to several cistrons indicate the presence of multicistronic transcriptional units. After the multicistronic unit has been defined by these complementation associations, the gene order in this unit was determined by assuming that Mu insertion in the first gene in the unit would prevent expression of all the cistrons in the unit, whereas insertion in the second gene in the unit would affect expression of all the cistrons except the first, and so on.

When it was necessary to move gene defects to the episome, as when an F *his*$^+$ *cheC* episome was required, the method previously described was used (14). To further characterize some Mu-induced mutations and to test the possibility that our collection of point mutations did not include representatives of all the flagellar genes in the map region, a procedure was developed to collect specific classes of episomal point mutations. For example, we sought episomes with point mutations which would not complement the Mu-induced *flaO* defects. To specifically collect these, 10 ml of cells in L broth ($\sim 2 \times 10^8$ cells/ml) of a donor strain MSF1338 (F1338 in strain JC1553) was mutagenized with 50 μg of *N*-methyl-*N*-nitro-*N*-nitrosoguanidine per ml in tris(hydroxymethyl)aminomethane (Tris)-maleate buffer, pH 6.0, for 30 min at room temperature. After this, the mutagenized donor strain was washed two times in Tris-maleate buffer, resuspended in L broth, and mated in approximately equal proportions with the desired Rec$^-$ Fla$^-$ recipient strain in L broth at 37 C for 1 h. The mating mixture was then washed in minimal medium and concentrated to 0.5 ml. These bacteria were then spread on several minimal medium plates which selected for His$^+$ exconjugants. After about 36 h at 37 C, the colonies ($\sim 10^3$ per plate), most of which were Fla$^+$ bacteria, were resuspended from the agar plate in L broth (minus glucose) and exposed to χ phage as in the χ-resistance selection procedure mentioned above (14). The χ-resistant bacteria in the overlay agar were streaked on minimal medium agar to get separate clones and were tested for motility. The immotile clones were found to contain mostly F *his*$^+$ episomes which now carried one specific *fla* point mutation, namely, the one sought which would not complement the chromosomal *fla* defect in the recipient strain. This technique was used to select *flaO* point mutations by picking strains that carried episomes that would not complement a new class of Mu-induced mutations. Eight episomes which carried *flaO* mutations were selected. Although episomes with *flaE* point mutations could also arise in this selection procedure, they were not found. This may be a result of the slight spreading that strains carrying *flaE* defects show on tryptone motility agar plates and of our selecting only those clones which showed no spreading. The F element in these merodiploid strains could be transferred to a variety of recipients to test

the nature of the episomal flagellar mutation.

P1 transduction and electron microscope examination were performed as in Silverman and Simon (14).

RESULTS

Location of flagellar defects. Twelve of the 240 strains originally isolated were discarded for reasons described in Materials and Methods. Of the 228 Mu lysogenic strains with defective flagellar functions subsequently examined, lesions in 82 were assigned to region III, between *his* and *uvrC* by F-element mapping. The lesions in 102 were assigned to region II, between *uvrC* and *aroD*. Most of the remaining 44 strains probably harbored lesions in region I between *pyrD* and *trp* and will be the subject of another report.

Complementation analysis. By using a donor master which contained strains donating F elements with a series of defined point mutations that mapped in regions II and III, it was possible to test many Mu-induced mutant strains. Fig. 1 shows the result of complementation tests for several mutant strains with lesions in region III, all of which show different complementation behavior. It can be seen that some of the mutations in these strains map in specific cistrons (i.e., *fla-1503* = *flaA*, *fla-1527* = *flaD*, *fla-1630* = *flaE*, and *fla-1617* = *hag*) while others appear to belong to more than one cistron

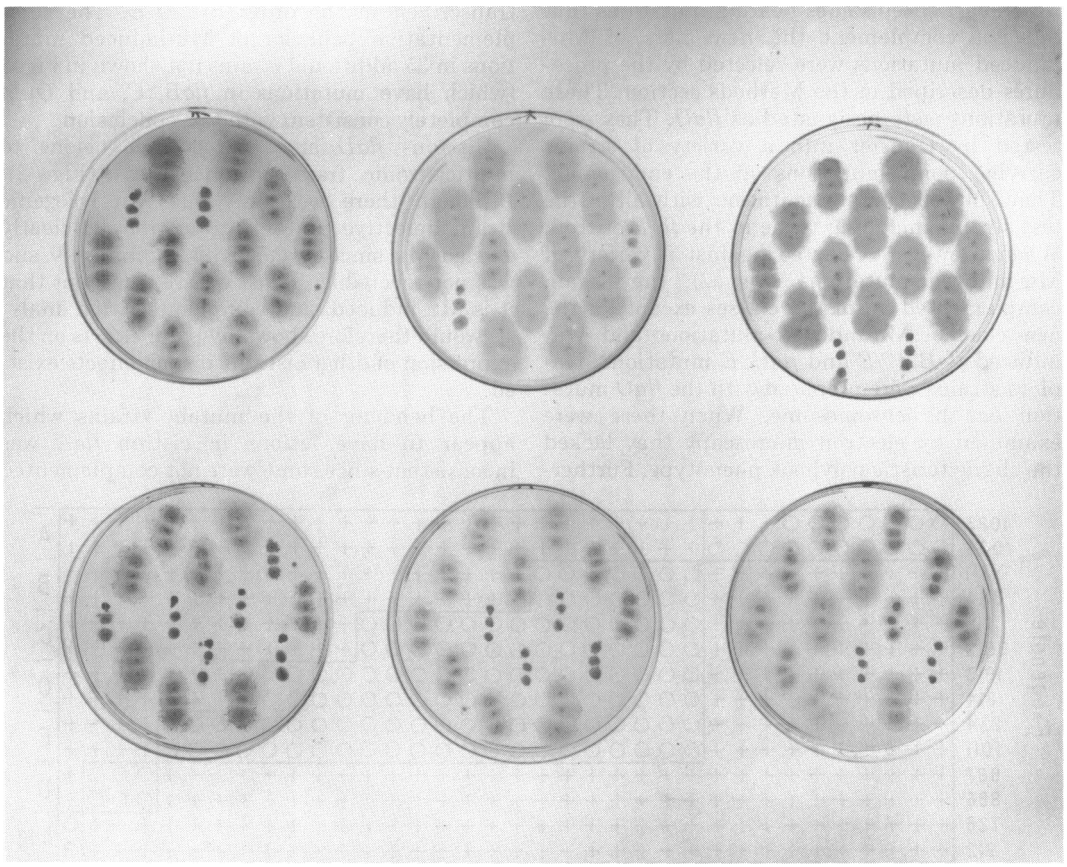

FIG. 1. *Complementation analysis of Mu-induced flagellar mutations. Each plate shows the capability for movement of 14 merodiploid strains constructed with identical region III Mu-induced flagellar defects on the endogenote and a series of defined point mutations on the exogenote. The endogenotes of the merodiploid strains on each plate are as follows: top row of plates, left to right, fla-1503, fla-1527, and fla-1617; bottom row of plates, left to right, fla-1509, fla-1515, and fla-1630. The 14 different genotypes on the exogenote are in the same position for each plate and are as follows (read left to right, top to bottom [5 rows]: (1) fla⁺ region III; (2) fla⁺ region II and III; (3) flaA1083; (4) flaA1004; (5) flaB111; (6) flaB394; (7) flaC101; (8) flaC8012; (9) flaD867; (10) flaD886; (11) flaE234; (12) flaE1011; (13) hag726; and (14) hag912. Movement through the minimal motility agar was interpreted as complementation (+), and lack of movement from the point of inoculation was interpreted as no complementation (0).*

(i.e., *fla-1509* = *flaB,C,E*; *fla-1515* = *flaC,E*).

In the course of the preliminary complementation test it became obvious that there was a group of mutants among the Mu-induced strains that we had not previously encountered. Initially they were classed together with strains with *flaE* mutations. However, when the putative *flaE* mutants were examined by electron microscopy they formed two groups, those that had the characteristic polyhook phenotype (13) and those that lacked it. In order to determine by further complementation tests whether there were two cistrons or simply two phenotypically different classes of *flaE* mutants, it was necessary to obtain specific point mutations. Strains which carried episomes bearing mutations that did not complement the new class of Mu-induced mutations were selected by the procedures described in the Methods section. These mutations were designated as *flaO*. They were tested by transfer into a variety of strains carrying point mutations on the endogenote. They showed complementation with all of the test strains including those in the *flaE* cistron. When they were retested against a variety of Mu-induced mutations (Fig. 2), they again complemented all of the classes except for the new class of Mu-induced mutations and Mu-induced *flaB,C,E* and *flaC,E* mutations. Haploid strains were prepared with the *flaO* mutation on the chromosome. When these were examined by electron microscopy they lacked the characteristic polyhook phenotype. Furthermore, they showed complementation when tested with *flaE* point mutations. The complementation behavior, as well as the phenotype of the Mu-induced mutations and the behavior of the point mutations, all argue for the existence of a new cistron *flaO*.

Examination of the 82 mutant strains with lesions in region III was carried out and Fig. 2 shows the results of some of the complementation tests. Since we found a class of mutants with mutations which behaved as *flaB*, *C*, *O*, and *E* lesions, a class which behaved as *flaCOE*, a class that behaved as *flaOE* lesions, and a class which behaved as *flaE* lesions, we concluded that a transcriptional unit composed of cistron *flaB*, *C*, *O*, and *E* exists which is transcribed in the order *B-C-O-E*. The complementation behavior of Mu-induced mutations in 35 additional strains not shown in Fig. 2 (which have mutations in *flaB*, *C*, and *O*) is completely consistent with this conclusion.

Cistrons *flaD* and *hag* do not belong to multicistronic transcriptional units (Fig. 2). Although there was only one Mu lysogenic strain defective in the *hag* cistron, it clearly carried Mu since it showed Mu immunity and was not revertible. Thus, we are confident that it is Mu induced, and complementation analysis would therefore show any polar effects on the expression of other cistrons if such effects existed.

The behavior of the mutant strains which appear to have lesions in cistron *flaA* was inconsistent since some were not complemented

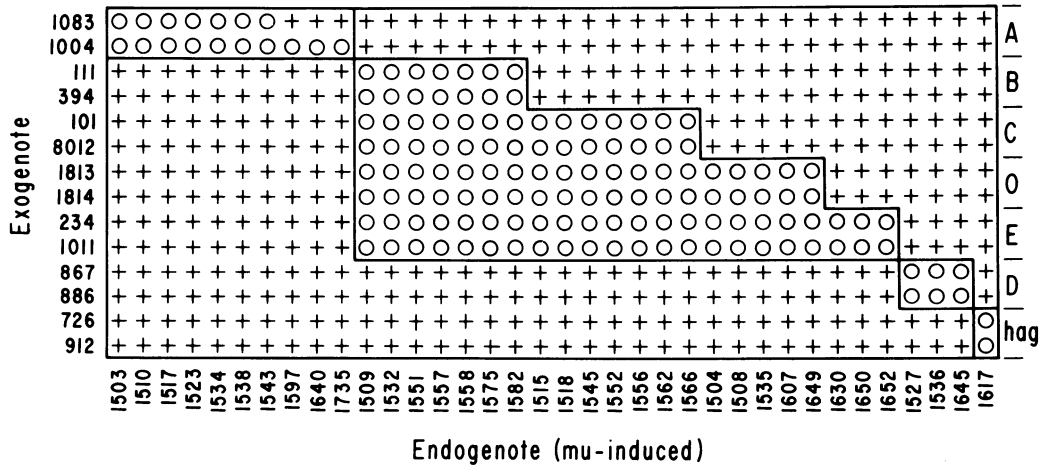

FIG. 2. *Summary of complementation analysis of strains containing Mu-induced flagellar defects which map in region III. Merodiploid strains were prepared with a defined flagellar point mutation on the exogenote (left column) and a Mu-induced flagellar mutation on the endogenote (bottom row). The F element carrying the point mutation was derived from F1334. Symbols: +, complementation; 0, no complementation. Letter in right column denotes the cistron to which the defined exogenote defect belongs (i.e., cistrons fla-1083 and fla-1004 belong to cistron A).*

by *fla-1083* (see Fig. 2). This suggested that *flaA* might consist of more than one cistron. This kind of complementation behavior is sometimes seen with point mutations and is called partial or intragenic complementation (8, 10). However, the complementation observed was "good," not "partial," and there is little reason to expect that Mu-induced mutations would produce a gene product with any activity since the integrity of the gene has been interrupted by prophage insertion. This led us to reexamine our initial interpretation of the complementation behavior of ethyl methanesulfonate (EMS)-induced *flaA* mutations (14). The complementation tests were repeated and the data was similar to that reported earlier (except with respect to the strain carrying mutation *fla-641*). The complementation relationships between EMS-induced point mutations in *flaA* can also be interpreted by resolving the group into more cistrons (Fig. 3). The complementation behavior of pairs of mutations in merodiploid strains formed by combination of *fla-371*, *fla-871*, *fla-1083*, and *fla-1004* on the exogenote with Mu-induced *flaA* mutations was unambiguous and therefore suggested four cistrons (see Fig. 3) which were designated *flaA*, *flaP*, *flaQ*, and *flaR* with the order of transcription *A-P-Q-R*. Cistron *flaA* was defined by *fla-371*, cistron *P* by *fla-871*, cistron *Q* by *fla-1083*, and cistron *flaR* by *fla-1004*. All of the Mu-induced *flaA*, *P*, *Q*, and *R* mutations are shown in Fig. 3 except one, *fla-1546*, which behaved as an *flaP* point mutation.

The division of *flaA* into cistrons *flaA*, *flaP*, *flaQ*, and *flaR* is further supported by the evidence that the *flaA* cistron and the *cheC* cistron defined by Adler (1-3) are identical. Table 1 shows that *cheC497* on the exogenote gave a complementation pattern which confirmed its identity with *flaA* and also with the mutant loci in nonchemotactic strains e20k1 and e21o2 which were isolated by J. S. Parkinson. These latter mutations show complementation with all other cistrons except *flaA* (not shown in Table 1). In addition, when *fla-371*, the point mutation that defines cistron *flaA*, was on the exogenote, it resulted in a pattern of complementation identical to *cheC497*. The merodiploid strain with *fla-371* on the exogenote and *cheC497* on the endogenote was not constructed because the recipient strain was Rec$^+$ and complementation analysis would have been difficult to interpret (see reference 14). These results indicated that the *cheC* mutations are members of the *flaA* cistron.

Of the 82 mutant strains with Mu-induced lesions in region III, one strain, MS1502, harbored a defect which was complemented by representatives of all known *fla* cistrons including *flaO*. Complementation analysis and deletion mapping in progress at this time will be necessary to determine whether this strain represents an additional cistron.

Complementation analysis of strains carrying Mu-induced mutations mapping in region II clearly show that cistrons *flaG* and *flaH* belong to a multicistronic unit which is transcribed in the order *flaG-flaH* (see Fig. 4). Cistrons *flaI* and *mot* do not appear to be organized into multicistronic units. Electron microscopic ex-

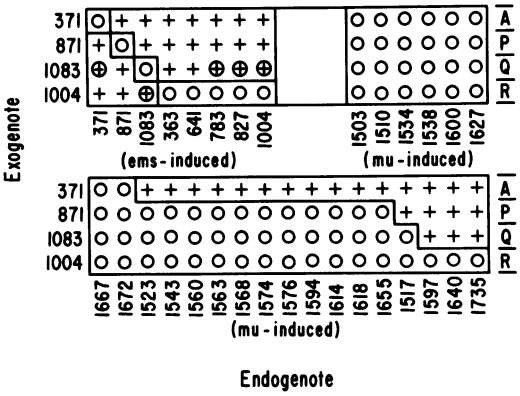

FIG. 3. *Summary of the complementation relationships of pairs of flaA mutations in merodiploid strains. The symbols are the same as in Fig. 2 except ⊕ = poor complementation. The letter designation in the right-hand column denotes the cistrons into which flaA was divided.*

TABLE 1. *Summary of the complementation relationships between mutations cheC497 and flaA371 and other mutations in region III*

Merodiploid		Complementation[a]
Exogenote	Endogenote[b]	
flaA371 or *cheC497*	*flaA371*, *e20k1*, *e21o2*, *flaA1503*, *flaA1510*, *flaA1534*, *flaA1538*, *flaA1600*, *flaA1621*	0
flaA371 or *cheC497*	*flaP871*, *flaQ1083*, *flaR1004*, *flaB915*, *flaB9910*, *flaC231*, *flaC814*, *flaE1011*, *flaE1071*, *flaD691*, *flaD886*, *hag726*, *hag912*, *flaP1523*, *flaQ1517*, *flaR1597*, *flaB1509*, *flaC1545*, *fla01535*, *flaE1630*, *flaD1536*, *hag1617*	+

[a] Symbols: 0, no complementation, +, complementation.
[b] Recipient strains with the endogenote genotypes are Rec$^-$.

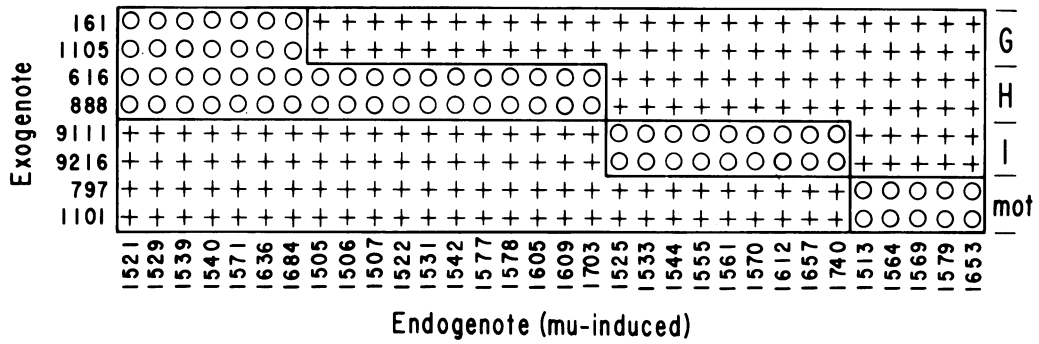

FIG. 4. *Complementation analysis of Mu-induced mutations in region II. Details of this analysis were the same as those shown in Fig. 2. The exogenote point mutations are carried on an F element derived from F1338. Letters in the right-hand column denote the cistron of the exogenote mutation.*

amination of the strains carrying Mu-induced *mot* mutations revealed the presence of apparently normal flagella. These flagella must be paralyzed since these strains showed no translational motion in liquid culture or on motility plates. Therefore, these strains which were classified by complementation analysis as carrying *mot* mutations have the phenotype of Mot⁻ strains.

P1 transduction. To improve our understanding of the orientation of cistrons in region II, the cotransduction data in Table 2 was obtained. It can be seen that cistrons *flaG* and *flaH* are mapped in a cluster and cistrons *mot* and *flaI* are located together between *flaG* and *flaH* and the *uvrC* locus. By using the mapping function developed by T. T. Wu (17), which relates cotransduction frequency to map intervals, the frequencies for region II flagellar loci were used to position them with respect to the *uvrC* gene. These data and the information defining cistrons in region III and II obtained earlier (14) are presented in Fig. 5 to give a summary of our current knowledge of the organization of the flagellar genes between *his* and *aroD*.

TABLE 2. *Region II: cotransduction of fla⁺ with uvrC*

Donor genotype[a]	Recipient genotype	Fla⁺/uvr⁺ [b]	
		Ratio	%
uvr⁺ fla⁺	*uvrC flaG161*	68/336	20
	uvrC flaG9812	80/336	23
	uvrC flaH616	94/336	28
	uvrC flaH705	86/336	26
	uvrC flaI851	170/336	51
	uvrC flaI1016	211/336	63
	uvrC mot92	174/336	52
	uvrC mot9413	182/336	54

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

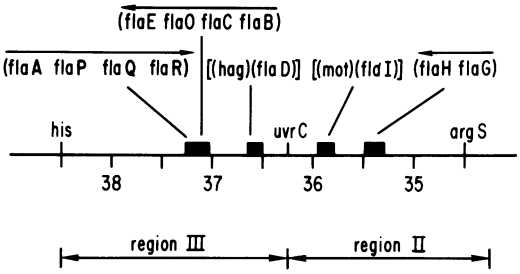

FIG. 5. *Organization of the flagellar genes in regions II and III on the E. Coli genetic map. Arrows indicate membership of and direction of transcription of cistrons in operons. Parentheses indicate uncertainty in orientation of the enclosed cistron or operon and brackets indicate uncertainty in the orientation of the enclosed cistrons.*

DISCUSSION

The use of merodiploid strains with defined flagellar mutations on the exogenote and Mu-induced flagellar mutations on the endogenote provides a reliable test of the organization of flagellar genes into multicistronic transcriptional units. There is a great deal of agreement between the mapping (i.e., cotransduction analysis and three-point crosses) and the grouping obtained from the complementation tests. For example, it was suggested that *flaB*, *C*, and *E* form an operon since we found previously that they were very close together (14) by using P1 transduction and by finding amber mutations which appeared to belong to both cistrons *flaB* or *C*. In this study, using Mu-induced mutations, it became readily apparent that cistrons *flaB* and *flaC* are in the same operon with *flaE* and *flaO*.

As a result of this study we have further refined the genetic map of the flagellar genes in the *his-aroD* region. What was once defined as *flaA* now appears to be a multicistronic unit

composed of four cistrons, *flaA*, *flaP*, *flaQ*, and *flaR* transcribed in the order *A-P-Q-R*. Examination of the complementation relationships of EMS-induced mutations supported this arrangement, but the analysis of Mu-induced mutations was especially convincing. Definition of this unit as four cistrons is further strengthened by the evidence which showed *flaA* to have an identity apart from all other cistrons; the *flaA* locus corresponds to the *cheC* gene described by Adler and Armstrong (1-3).

flaG and *flaH* belong to a unit transcribed in the order *flaG-H*. Cistrons *hag*, *mot*, *flaI*, and *flaD* do not appear to belong to multicistronic transcriptional units. Although only one strain with an Mu-induced *hag* mutation was found in 228 strains, it did not show polar effects and it does not map adjacent to other *fla* cistrons (14), and we therefore conclude that the *hag* gene is in a separate grouping. Of 77 Mu-induced *flaB*, *C*, *O*, *A*, *P*, *Q*, or *G* mutations examined, one, an *flaP* mutation, behaved as a nonpolar point mutation. It might be noted that as many as 13% of Mu-induced mutations in the *lacZ* gene were deletions (6). The presence of deletions in the flagellar genes in this study might exaggerate our impression of the extent of the membership of cistrons in multicistronic transcriptional units. However, these deletions should be a minority of the mutants and, in any case, could show up in the analysis as inconsistencies in complementation relationships. For example, some mutations might be found which appear to belong to both the *flaA*, *P*, *Q*, *R* cistrons and the *flaB*, *C*, *O*, *E* cistrons. These types were not seen.

The frequency of Mu-induced mutations in various regions of the *E. coli* map and in various cistrons in these regions diverged somewhat from the frequencies obtained with another mutagen (EMS). For example, only one *hag* mutation was found, more mutations in the *flaA*, *P*, *Q*, *R*, cistrons were found, and there were proportionately more mutations found to reside in region II. However, the same relatively large number of *flaB* and *flaC* mutations were found as with EMS. Some of these differences may reflect the different mechanisms of mutagenesis.

One, and possibly two, new cistrons were defined by this study. How many remain unrecognized? We have classified the mutations in a total of 191 strains into 14 cistrons in the *his-aroD* region. If the distribution of mutations in cistrons was close to random, it would be possible to estimate the probability of not having defined a cistron with a mutation. However, the distribution of mutations if far from random. The class of *flaB* and *C* mutation is very large, whereas the class of *flaQ* mutations has two members. Therefore, it can not be said with certainty that we have accounted for all the flagellar genes in the *his-aroD* region.

The organization of many of the flagellar genes into multicistronic units may reflect a solution to the problem of regulating the synthesis of the many components needed for the assembly of flagella. The assembly requires greatly differing contributions in terms of the number of each kind of structural protein molecule. For example, a typical flagellum might require 10,000 flagellin molecules, 50 to 100 hook molecules, and 1 to 50 of the particular molecules needed for the basal structure. By organizing the flagellar genes into multicistronic units, the amounts of certain gene products could be coordinately controlled. By organizing a flagellar gene into a transcriptional unit of one cistron, the amount of a gene product could be controlled independently of the level of expression of other proteins. Interestingly, the *hag* gene which codes for the structure of the flagellin molecule appears not to be transcribed in a multicistronic unit. Since, as far as we know, no other flagellar gene product is required in amounts approaching that of flagellin, it is reasonable that this gene should be transcribed independently. Of course, other regulatory mechanisms such as ones at the translational level are conceivable but we have no evidence as yet for them.

Superficially there is a reasonable agreement between the genetic distribution of the flagellar genes in *E. coli* and in *Salmonella* (18). Interspecific mating can now be used to analyze the degree of homology between the genetic structure and regulatory mechanisms in those organisms.

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