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Positioning Flagellar Genes in *Escherichia coli* by Deletion Analysis

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Two methods were devised to select a series of overlapping deletion mutations carried on episomal elements in *Escherichia coli*. The deletions were then used in an analysis of (i) the relative position on the genome of previously described mutant loci in the flagellar genes, (ii) the relative position of a newly defined cistron, *flaN*, and (iii) the orientation and direction of transcription of genes previously assigned to multicistronic transcriptional units. As a result of this analysis and previous work, we report the following arrangement of the flagellar genes in the *his-aroD* region of the *E. coli* genetic map: *his-flaR-flaQ-flaP-flaA-flaE-flaO-flaC-flaB-flaN-hag-flaD-uvrC-flaI-mot-flaG-flaH*. The genes *flaA*, *P*, *Q*, and *R*, *flaB*, *C*, *O* and *E*, and *flaG* and *H* are co-transcribed in that order.

Information about the organization of the genes necessary for the assembly and function of the *Escherichia coli* flagellum may be valuable in determining how the expression of these genes is regulated. At least 14 genes necessary for flagellar assembly and function thus far have been defined by complementation analysis in *E. coli* (6, 7). Genetic analysis of the polar influence of Mu phage-induced mutations indicated that many flagellar genes are organized into multicistronic transcriptional units, whereas others are transcribed singly (7). While mapping by transduction and F elements established the position of some groups of genes, a more precise location and orientation was sought for them by using deletion analysis.

This investigation reports the methods that were devised to obtain mutations which deleted the flagellar genes. A series of overlapping deletions were selected on F elements which carried the flagellar genes in the *his-aroD* region of the *E. coli* chromosome. These F elements were used to form merodiploid strains with the deletion on the exogenote and a defined point mutation on the endogenote. The extent of the deletion could be mapped by testing the ability of the merodiploid strain to produce the Fla^+ phenotype. From this series of overlapping deletions, the arrangement of most of the genes in the *his-aroD* region was obtained.

Analysis of the complementation behavior of flagellar mutations in some strains suggested the presence of a previously undefined gene, *flaN*.

The arrangement of the flagellar genes in the *his-aroD* region of the *E. coli* chromosome was determined to be: *his-flaR-flaQ-flaP-flaA-flaE-flaO-flaC-flaB-flaN-hag-flaD-uvrC-flaI-mot-flaG-flaH-aroD*.

The phenotype of merodiploids constructed in *recA* strains with the deletion mutation on the exogenote and a defined point mutation on the endogenote indicated whether complementation occurred between the mutant loci. Therefore, not only the extent of the deletion but the influence of the deletion on the expression of intact genes could be measured. Some deletions did exert polar influences on the expression of other genes and the nature of these effects suggested the existence of operons composed of several genes. The results of this study further demonstrated the presence of three multicistronic transcriptional units and confirmed the conclusions reached from previous genetic analysis of polar Mu phage-induced flagellar mutations (7). These multicistronic transcriptional units are: the *flaA*, *flaP*, *flaQ*, *flaR* unit; the *flaB*, *flaC*, *flaO*, *flaE* unit; and the *flaG*, *flaH* unit with transcription proceeding in the order given.

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.

Bacterial strains. The *E. coli* K-12 strains used in this study for the selection of F elements carrying deletion mutations and for the complementation and

recombination analysis required to characterize these deletions have been described elsewhere (6).

The selection of deleted F elements took place using strains JC1553 or MS1032 carrying F element F1829. Strain JC1553 carries the following markers: *leu, his, argG, met, str, fla*$^+$, *lac, mal, xyl, mtl, recA*. Strain MS1032 carries *thr, leu, Bl, his, argE, pro, str, lac, gal, ara, xyl, mtl, recA, fla*$^+$, and *hag-208*. Complementation analysis required the formation of merodiploid strains with a deletion mutation on the exogenote and a defined point mutation on the endogenote. The recipient strain with the endogenote mutation was always, unless otherwise noted, a Rec$^+$ or Rec$^-$ derivative of strain MS1350 which has the genotype: *his, thy, argE, uvrC, galU, sup*$^+$, *str, fla*$^+$, and *hag-207*. The *hag* gene designation refers to the antigenic nature of the flagellin molecule produced (see 6). The endogenote mutations used for the characterization of the F element deletions are located in strains which have been described previously (6). The *cheC497* mutation is in strain AW405 where the relevant markers are: *thr, leu, Bl, his*. The nonchemotactic phenotype of this strain has been described by Armstrong and Adler (1, 2).

Selection of deletion mutations. The mutations selected for this study were deletions in F element F1829 which was derived from F element F1338 described previously (6). F1338 carries the part of the chromosome that includes the *his* marker, the *fla* genes, *uvrC* and extends past the *zwf* marker. Most of the genes that control the synthesis of flagella were found on this part of the chromosome. Region III refers to those flagellar genes located between *his* and *uvrC* and region II refers to those flagellar genes located between *uvrC* and *aroD* (Fig. 5). F1829 differs from F1338 in that it carries a mutation, *fla1829*, which renders flagellar synthesis insensitive to catabolite repression. It was prepared in a Crp$^-$ derivative of strain MS1350. One consequence of the Crp$^-$ phenotype results in the inability to synthesize flagella. A series of motile revertants were selected and only those that reacquired motility but still maintained other characteristics of the Crp$^-$ phenotype (i.e., the inability to utilize maltose or lactose) were saved. A mutation from one of these strains, *fla1829*, was subsequently transferred to the episome and the episome was put into a number of different strains. The episome carrying *fla1829* allows wild-type cells to make flagella in the presence of high levels of catabolites. It also restored the ability to make flagella of strains that are defective in adenyl cyclase (9). A full characterization of this *fla1829* mutation will be presented elsewhere.

Selection of type 1 deletions. A series of deletions entering the flagellar genes in region III (between *his* and *uvrC*) from the *his* direction were isolated from F element F1829 in strain JC1553 in the following manner. Strain JC1553 harboring F element F1829 was inoculated in a narrow zone across the center of a motility agar plate containing 5 g per liter D-glucose, D-gluconate, and D-mannitol, and motile (Fla$^+$) swarms of bacteria were selected. The strain will not swim through this agar because, in the presence of the three carbon sources, it produces large quantities of mucopolysaccharide which interfers with motility (see 6). Any of the strains that we have used that are diploid for the genes in region III overproduce this mucopolysaccharide in the presence of glucose. Mucopolysaccharide production decreases when the cells are grown on minimal medium with glycerol. The nature of this effect is unclear, but it has been observed (6; J. Parkinson, personal communication) that if a locus in region III is deleted, the abnormal production of mucopolysaccharide ceases and the merodiploid strain will swim through motility agar. (Introduction of a *galU* mutation will also prevent this mucopolysaccharide production. The recipient strains used in the recombination and complementation analysis are GalU$^-$ to prevent this from affecting flagellar synthesis.) Therefore, one way to obtain F element deletions is to require the merodiploid to swim through motility agar which includes glucose, gluconate and mannitol to maximize mucopolysaccharide production. All of the motile swarms should be nonmucoid and many should harbor F element deletions. Segregation and loss of the entire episome is prevented since the *fla1829* alteration on the episome must be retained to insure flagellar synthesis under catabolite repressing conditions. This selective pressure for both the deletion of a locus on the F element and retention of another locus on the F element resulted in a series of strains which harbored F elements with deletions extending into the flagellar genes in region III. Since, as will be reported later (Silverman and Simon, manuscript in preparation), *fla1829* is located in region II proximal to *uvrC*, these deletions never extended into region II because the loss of *fla1829* was proscribed.

Strains collected by this procedure were streaked and cloned on minimal agar minus histidine to insure that the episomal *his*$^+$ genes were retained since later F element transfer required this property. F element deletions isolated in this manner will be referred to as type 1 in the text to follow.

Selection of type 2 deletions. Another method was devised to select F elements with deletions originating in region III with no restrictions on the extent of deletion into region II. This selection is based on the observation (M. Silverman, Ph.D. thesis, UCSD, 1972) that in strains that are diploid for the *hag* gene, carrying one form (e.g., *hag207*) on the endogenote and another form on the episome (e.g., *hag208*) the resulting flagella are mixtures of both types of subunits and react uniformly with both types of antibody. Strain MS1032 harboring F element F1829 was inoculated in a narrow zone across the center of a minimal agar motility plate containing all the necessary nutritional requirements except histidine and containing a 1/2,000 dilution of sterile anti-Hag207 antisera, and motile (Fla$^+$) swarms of bacteria were selected. Since the strain will normally be immobilized by anti-Hag207 antisera, motile (Fla$^+$) bacteria would have to (i) retain the *his*$^+$ gene on the F element and (ii) delete or inactivate the *hag207* episomal gene. Bacteria with *hag208* flagella (strain MS1032) will swim through agar containing anti-Hag207 antiserum. Figure 1 shows that motile swarms arise when the above selective procedure is applied. Most of the strains selected in this manner were found to harbor abbreviated F elements. All F elements which could be shown

to have deleted flagellar genes had as a minimum deleted the *hag207* locus. Only two strains isolated appeared to have acquired *hag* gene point mutations. Of the strains with deleted F elements, many were still mucoid on minimal agar containing glucose. Thus, these deletions did not extend into the genes which map between *his* and *fla* (region III) and are involved in producing the mucoid effect. F elements isolated in this manner will be referred to as type 2 in the text. An additional F element derived from F1338, F1817, was included in the analysis. It carried a spontaneous deletion found in the course of a selection procedure used for collecting specific episomal point mutations (7).

Characterization of extent and polar influence of F element deletion. The various deletion mutations carried on F elements were characterized by transferring the F element from the donor His^+ merodiploid strain JC1553 or MS1032 into recipient bacteria with defined flagellar point mutations. As shown in Fig. 2, this transfer was accomplished by mating the donor strain on a lawn of certain Rec^+ or Rec^- recipient bacteria spread on an L agar plate. The donors were transferred from a minimal agar plate (– His) with a sterile toothpick and inserted into a position on the L agar mating plate (first column of plates in Fig. 2). After 6 h of incubation at 37 C, a sterile toothpick was inserted into the position where the donor and recipient bacteria were in contact and the mating bacteria were transferred to a minimal agar plate selecting for His^+ recipient exconjugants and counterselecting both donor and recipient bacteria by amino acid deprivation (second column of plates in Fig. 2). After incubation for 24 h at 37 C, a sterile toothpick was used to transfer His^+ exconjugant bacteria to a minimal motility agar plate which selected for His^+ exconjugant bacteria. Movement on this plate indicated that a nondefective flagellar genotype had resulted by recombination or that exogenote and endogenote flagellar defects had complemented (see third column of plates in Fig. 2). Initially, F elements were mated with representatives of several different flagellar genes in Rec^+ and Rec^- strains.

The complementation relationships reported here for the *flaN* gene were obtained from matings performed as above except RecA recipient bacteria were used. In contrast to the analysis performed with Rec^+ diploid bacteria where recombination was possible to produce a nondefective flagellar genotype, movement of the Rec^- merodiploid strains constructed with a deletion mutation on the exogenote and a defined point mutation on the endogenote indicated that complementation, rather than recombination, had occurred. Therefore, the polar influence of a deletion mutation on the expression of a nondefective gene could be measured in Rec^- merodiploid strains.

RESULTS

Order of the flagellar genes. F elements carrying deletions were tested by checking for their ability to form recombinants in a variety of strains carrying flagellar point mutations. Each deletion was tested against all the mutants listed in Fig. 3. Eighty-two strains with F

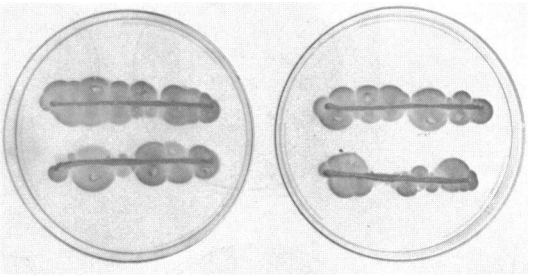

FIG. 1. *Isolation of strains harboring F element deletions. These petri plates contain minimal motility agar plus anti-Hag207 antisera. The swarms of bacteria moving from the zone of inoculation were tested for the deletion of the episomal hag207 locus as well as the other fla loci.*

elements were used. Not all of the results are shown since many were redundant. The order that results is: *his-(flaR, flaQ, flaP) flaA (flaE, flaO) flaC, flaB, flaN, hag, flaD, flaI, mot, flaG, flaH-aroD*. The order of the genes in parenthesis is not apparent from this analysis. However, previous work with Mu induced mutations suggests the order presented in Fig. 3. It should be noted that the representation of the terminus of the deletion shown in Fig. 3 means that the deletion ends between the mutant loci noted rather than at the end of the gene as shown. In some cases, the deletion does extend part way into the adjacent gene.

Previously, we placed the *cheC497* mutation in the same gene as *flaA* mutations based on its complementation behavior. To further check this assignment, *cheC497* was tested by deletion analysis. It is indistinguishable by this technique from *flaA371*.

Evidence for a cistron flaN. Strains with the *flaN* gene defect included in this study were collected recently and have a Fla^- genotype. One *flaN* strain was isolated as a Mu phage induced flagellar mutant (7) and subsequently other *flaN* point mutations were isolated. The complementation relationships which defined this gene are shown in Table 1. *Fla1502* is a Mu phage induced mutation (7) and the other *flaN* mutations (*fla1810* and *fla1812*) were isolated originally as NTG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) induced episomal point mutations selected for their inability to complement *fla1502* (7). These point mutations were then transferred by P1 transduction to strain MS1350 by selection for $UvrC^+$ transductants as previously reported (6). The complementation analysis which had to be performed in RecA merodiploid strains shows that *fla1810* and *fla1812* failed to complement only *fla1502*, *fla1810*, and *fla1812* and did complement point mutations representing all other previously defined flagel-

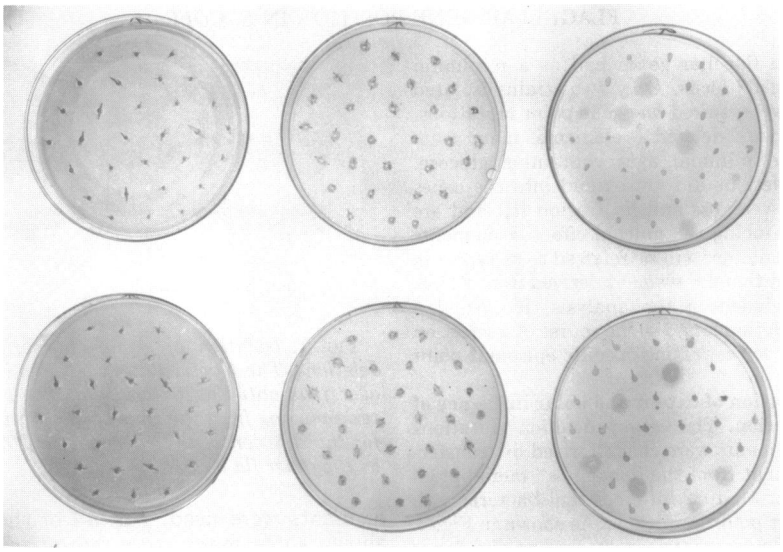

FIG. 2. *The extent of deletion of F elements as determined by recombination with defined flagellar point mutations. First column of plates: donor strains harboring F element with deletion mutations (30 insertions per plate) are mated on a lawn spread with one Rec⁺ recipient strain carrying a defined flagellar point mutation. Second column of plates: minimal agar plates which select for His⁺ exconjugant merodiploid bacteria from matings on first plate. Third column: minimal motility agar plates on which the flagellar phenotype of the His⁺ exconjugant merodiploid bacteria is tested. Diffuse zone of growth indicates motility of His⁺ exconjugant bacteria.*

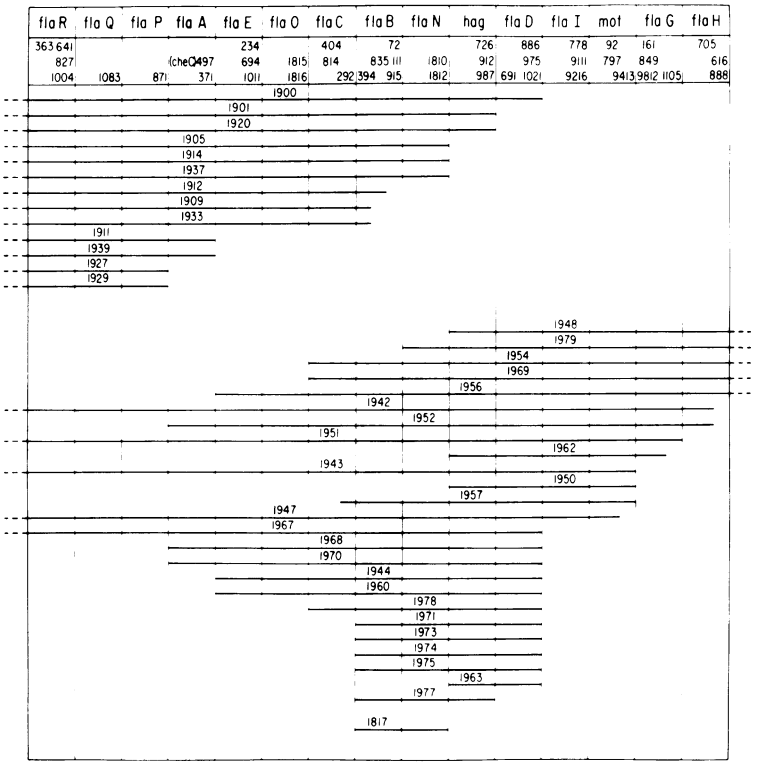

FIG. 3. *Characterization of F element deletion mutations. The extent of a particular deletion mutation is indicated by the heavy horizontal line which refers to the flagellar loci noted directly above in the second row. These flagellar loci define the cistrons noted directly above in the first row. The defined flagellar mutations are arranged so that the deleted regions are contiguous. The dotted heavy line indicates that the deletion continues outside the flagellar gene region.*

TABLE 1. *Complementation relationships between flagellar mutations in RecA merodiploid strains*

Merodiploid		Complementation[a]
Exogenote defect	Endogenote defect	
fla1810, fla1812	*fla1810, fla1812, fla1502*	0
flaA371, flaP871 flaQ1083, flaR1004 flaB111, flaB394, flaC101 flaC8012, flaD867, flaD886 flaE234, flaE1011 flaO1815, flaO1816 hag726, hag912	*fla1810, fla1812, fla1502*	+
fla1810, fla1812	*flaA371, flaP871 flaQ1083, flaR1004 flaB915, flaB9910 flaC231, flaC404 flaD886, flaD1021 flaE234, flaE694 flaE1011, flaE1071 flaO1815, flaO1816 hag726, hag912*	+

[a] +, Complementation; 0, no complementation.

lar genes as well as Mu phage induced mutations representing all known flagellar genes in region III (data not shown). Furthermore, point mutations representing all previously defined flagellar genes in region III carried on F elements complemented the endogenote flagellar defects *fla1502*, *fla1810*, and *fla1812*. Therefore, *fla1502*, *fla1810*, and *fla1812* are mutations in one gene and this gene is not one previously defined. The deletion analysis indicated a location for mutations *fla1810* and *fla1812* separate from the locations of other previously defined flagellar genes. These data justify the definition of a separate cistron, *flaN*.

Distribution of deletions. A large number of deletions were tested. Table 2 shows the distribution of the termini of these deletions. A total of 728 F elements were tried and only a fraction were useful for examining the positions of the flagellar genes. Among 417 type 1 F elements, 216 deleted none of the genes in region III and 165 deleted all of region III. Among 311 type 2 F elements, most deleted all the flagellar genes in region III and region II. If the remaining deletions are assumed to occur randomly along the chromosome, then the distribution of their termini can be explained as being proportional to the map distance in the interval between flagellar genes. Thus, many of the deletions terminate between *flaD* and *flaI*, between *flaN* and *hag*, and between *mot* and *flaG*. These regions correspond to the places along the genetic map that separate clusters of flagellar genes (6, 7). No type 1 F element carried a deletion that extended into region II. This is probably due to the restriction imposed by the selection procedure.

Complementation tests with deletions. Examination of *RecA* merodiploid strains with a deletion mutation on the exogenote and a defined point mutation on the endogenote measured the ability of these defects to complement each other. We sought to measure the influence of deletion mutations on the expression of nondefective exogenote genes. Figure 4 summarizes the information obtained by analyzing the movement of *RecA* merodiploid strains on motility agar. The solid line indicates that absence of complementation of a particular deletion with defined endogenote point mutations. The arrows mark the approximate end points of the deletions. It is apparent that some deletions affect the expression of nondefective genes. For example, mutation *fla1962* deletes part of the *flaG* gene but the expression of *flaH* is prevented. Likewise, deletion into *flaA* prevents the expression of *flaP*, *flaQ*, and *flaR* (see deletions *fla1956*, *fla1952*, *fla1960*) and deletion into *flaB* or *flaC* (deletions: *fla1954*, *fla1978*, *fla1971*, *fla1975*, *fla1977*, *fla1817*) prevents the expression of *flaC*, *flaO* and *flaE*. Deletion of *flaR*, *flaQ* and *flaP* does not affect the expression of *flaA* (deletions: *fla1927*, *fla1929*). From results

TABLE 2. *Initial characterization of deletion mutations*

Interval	Episomal deletion			
	Type 1[a]		Type 2[b]	
	Left terminus	Right terminus	Left terminus	Right terminus
his to *flaR1004*	201	0	196	0
flaR1004 to *flaA371*	0	2	3	0
flaA371 to *flaE1011*	0	2	3	0
flaE1011 to *flaB72*	0	3	9	0
flaB72 to *flaN1810*	0	0	1	0
flaN1810 to *hag726*	0	23	7	0
hag726 to *flaD886*	0	6	0	0
flaD886 to *flaI778*	0	165	0	22
flaI778 to *mot797*	0	0	0	0
mot797 to *flaG161*	0	0	0	40
flaG161 to *flaH616*	0	0	0	9
flaH616 to *aroD*	0	0	0	148

[a] Type 1 F elements (216) checked that had no terminus in the *fla* region and are not included in the table.

[b] Two type 2 F elements appeared to have short deletion or point mutations in the hag gene and are not included in this table as are 90 other type 2 F elements which were tested for the right terminus of the deletion only.

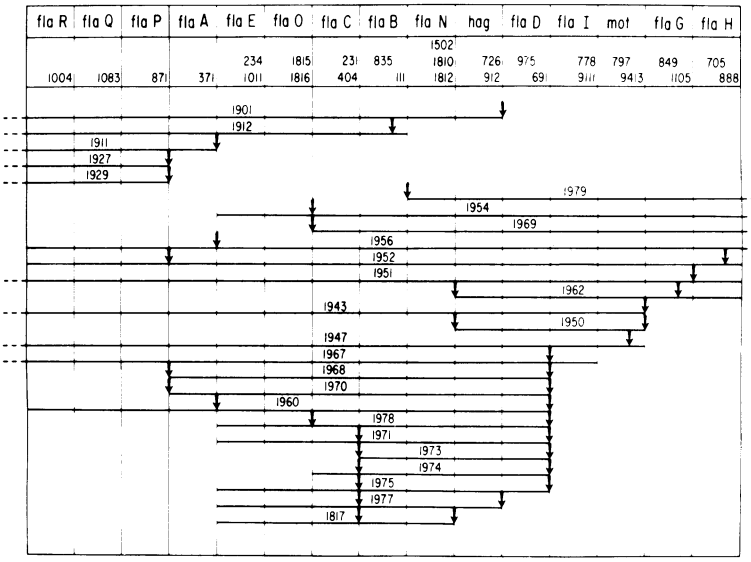

FIG. 4. *Characterization of the polar influence of F element deletion mutations. The details of this analysis are the same as in Fig. 3 except defined flagellar mutations are in RecA strains. The heavy horizontal line refers to the defective flagellar loci which are not complemented by the deleted F elements. The arrows show the extent of deletion as determined in Fig. 3.*

such as these, it is apparent that there exists a *flaG*, *flaH* (see deletions *fla1951*, *fla1962*) operon with *flaG* transcribed first, there is a *flaB*, *flaC*, *flaO*, *flaE* operon with *flaB* transcribed first, *flaC* second, and a *flaA*, *flaP*, *flaQ*, *flaR* operon with *flaA* transcribed first. The available deletions indicate that *flaN* is transcribed as a single gene operon but not all the tests could be done to determine if *flaD* and *hag* or *flaI* and *mot* are transcribed as part of an operon. None of these results suggest that they are. F element deletion *fla1967* apparently extends into the *flaI* gene, *flaI* function is lost but not *mot* gene expression. This confirms the order given in Fig. 3 for *flaI* with respect to neighboring genes.

It is noteworthy that some deletions, for example, *fla1968* and *fla1970* which extend into the *flaA* gene, do not affect expression of the *flaP*, *flaQ*, or *flaR* functions. *Fla1969*, *fla1973*, and *fla1974* also extend into the *flaB* or *flaC* gene but do not eliminate the *flaE* and *flaO* functions. Interpretation of these results will be made in the Discussion section.

Figure 5 represents a summary of the results presented in this paper and previous data (6, 7) presented on the organization of the genes that control flagellar formation.

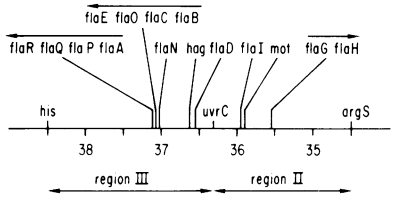

FIG. 5. *Summary of the position of flagellar genes on the E. coli genetic map. The arrows above groups of cistrons indicate organization of these cistrons into multicistronic transcriptional units with transcription in the direction shown by the arrows.*

DISCUSSION

The deletion analysis both confirms and extends our previous studies (6, 7). This analysis shows that the order of the genes in region III is *his-(flaR, flaQ, flaP) flaA (flaE, flaO) flaC-flaB-flaN-hag-flaD-flaI-mot-flaG, flaH-aroD.* In addition, the data confirm our previous finding that *flaA*, *flaP*, *flaQ*, and *flaR* form a polycistronic transcriptional unit and that *flaB*, *flaC*, *flaO* and *flaE*, and *flaG* and *flaH* also form such units. Most of the deletions that remove the first genes in these units also inactivate the rest of the unit. This is true for two deletions that we tested in the *flaG*, *flaH* group and for three in the *flaA*, *flaP*, *flaQ*, *flaR* group and six in the *flaB*, *flaC*, *flaO*, *flaE* group. However, two deletions of the *flaA* gene still leave expression of *flaP*, *flaQ*, and *flaR* and three deletions into *flaB* and *flaC* result in residual expression of *flaO* and *flaE*. These results are most consistently explained if we assume that in the case of the deletions that the remaining genes are now fused to another controlling unit and can therefore be expressed to some extent. This is precisely the situation that was found in an analo-

gous study of the complementation behavior of deletions into the lactose operon (4). More than 20% of the deletions that extended into the Z gene had residual permease and transacetylase activity. The residual activity was under the control of the purine operon to which these genes were fused (4). There are many other examples of such fusions (3, 5). This kind of deletion may be useful in further exploring the flagellar system. If the nature of the controlling element can be determined, it may be possible to derepress the synthesis of individual components of the flagellar system and perhaps isolate and characterize them biochemically.

The data presented further defines a mutant described earlier (7) that did not fit into the known complementation groups. It represents a previously unrecognized gene, *flaN*. The deletion map indicates that *flaN* is located between *flaB* and *hag*. The frequency with which deletions terminate in the *flaN-hag* and the *flaB-flaN* intervals suggests that *flaN* is very close to *flaB*. Further studies of recombination frequencies will be necessary to determine its precise position.

There is obvious superficial resemblance between the distribution of these genes in *E. coli* and the reported map in *Salmonella* (8). Interspecific crosses will be necessary to determine the precise degree of homology.

The results of this study permit a refinement of the genetic map for the flagellar genes in the *his-aroD* region. Figure 5 shows our current knowledge of the position and transcriptional organization of these flagellar genes. This knowledge, together with the information we are accumulating about the functions of the products of these genes, should permit a better understanding of the mechanism responsible for the regulation of the synthesis and assembly of the components of this organelle.

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