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Identification of the Structural Gene for the Hook Subunit Protein of *Escherichia coli* Flagella

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Previous studies showed that the structural gene for the flagellar hook subunit protein (molecular weight 42,000) was one of a group of flagellar genes located on the *Escherichia coli* genome near *pyrC*. Several lines of evidence indicate that the *flaK* gene is the structural gene for the hook subunit protein. Fla^+ strains that were insensitive to χ infection could be isolated as revertants of an $FlaK^-$ amber mutant strain but from no other Fla^- strain. The hook subunit proteins isolated from such χ-insensitive revertants of the $FlaK^-$ strain were shown to be antigenically and electrophoretically different from the hook protein isolated from the wild-type strain. Thus, reversion of a mutation in the *flaK* gene resulted in alteration of the structure of the hook protein. Furthermore, in programming experiments with hybrid λ containing flagellar genes, λ*fla* with *flaK* genetic activity programmed the synthesis of a 42,000-molecular weight protein, whereas λ*fla* without *flaK* genetic activity did not.

The flagellar organelle is responsible for motility and chemotaxis. The organelle has been purified from the cell envelope of *Escherichia coli* and consists of three morphologically distinct parts: the filament, the hook, and the basal structure (3, 4). The filament is composed of a single polypeptide subunit called flagellin with a molecular weight of 54,000 (10). The hook is composed of a single polypeptide subunit with a molecular weight of 42,000 (4, 20). The flagellar filament rotates, and the hook may act as a flexible universal joint connecting the filament to the basal structure anchored in the cell envelope. The basal structure is complex and is composed of at least nine different polypeptides (4). Other polypeptides necessary for flagellar formation and function are located in the cell membrane and cytoplasm (24, 25).

Genetic analysis of flagellar mutants has revealed the existence of at least 30 genes directly required for different aspects of flagellar function and formation (25). Some of these genes (*fla* and *hag*) program the synthesis of the filament, hook, and basal structure and regulate the appearance of these structures (4, 20, 22). The *mot* genes code for the synthesis of polypeptides, which are found in the inner membrane and appear to be specifically involved in flagellar rotation. The *che* genes program the synthesis of polypeptides that are necessary for the integration of signals from chemoreceptors and the modulation of the flagellar motion to produce responsive swimming. The *che* gene products are found in the cytoplasm and inner membrane of the cell, although some *che* gene products (i.e., *cheC*) may be a part of the flagellar rotor.

Until recently, little was known of the function of most of the flagellar genes. By using hybrid λ or plasmid vehicles carrying flagellar genes, it has been possible to determine the polypeptide gene products of most of the *mot* and *che* genes (17, 18, 23, 24). In addition, some of the genes associated with structural components of the organelle were defined by transferring a hybrid plasmid containing the *fla* genes in region I (Fig. 1) to a minicell-producing strain. Polypeptides synthesized in minicells purified from this plasmid-containing strain were programmed by the flagellar genes on the hybrid plasmid and were identical to several polypeptides known to be part of the hook-basal structure complex (11). The hook subunit polypeptide (molecular weight 42,000) was encoded by one of the genes in flagellar region I. Recent genetic analysis of region I flagellar mutants has refined our understanding of the complexity of the region. There are at least six flagella-related genes in this region: *flaV, flaK, flaL, flaM, flaS*, and *flaT* (9). In this report, we present evidence that *flaK* is the structural gene for the hook subunit protein.

MATERIALS AND METHODS

Media. Tryptone broth contained (per liter of distilled water): tryptone (Difco), 10 g; and 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 and tryptone agar plates were

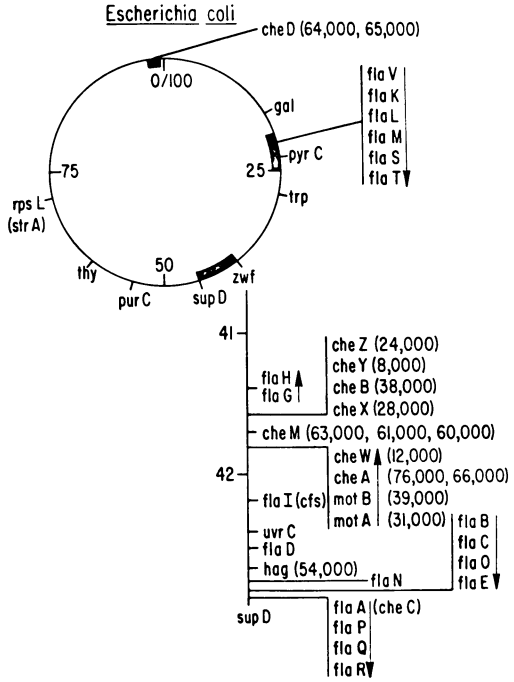

FIG. 1. *Location of flagellar genes on the E. coli genome. Flagellar genes are clustered in three regions: region I near pyrC; region II between zwf and uvrC; and region III between uvrC and his. The relative orientation of flagellar genes in region I with respect to pyrC is not known.*

prepared by adding 1.5% agar (Difco) to L-broth and tryptone broth, respectively. Motility plates were prepared by adding 0.35% agar to tryptone broth with 0.1 g of thymine per liter. Overlay agar was prepared by adding 0.4% agar to tryptone broth. Overlay agar contained 1.25 g of $MgSO_4 \cdot 7H_2O$ per liter.

Minimal medium for ultraviolet (UV)-irradiation experiments contained (per liter of distilled water): NH_4Cl, 1 g; KCl, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; Na_2HPO_4, 5 g; and KH_2PO_4, 2.4 g. After autoclaving this solution of salts for 15 min, the following sterile ingredients were added: 0.3 ml of 10^{-2} M $FeCl_3$, 30 ml of 50% glycerol, and 1.0 ml of 38% maltose. Incubation was at 37°C for the growth of bacteria and phage.

Bacterial strains. *E. coli* K-12 strains used in this study were derived from strain YK102 (*thi pyrC his argE thyA nalA uvrC mtl rpsL*) (9) and are described below.

Phage techniques. The test for growth of the flagellotropic phage χ on flagellar mutants was carried out as follows: a portion (0.1 ml) of exponential-phase culture (5×10^8 cells per ml) of the strain to be tested and 0.1 ml of χ suspension (strain XJ1 from T. Icho) were plated with tryptone broth overlay. After incubation for 8 h, the overlay was homogenized by pipetting and spun down by centrifugation, and the resultant supernatant was examined for χ-phage titer. For titration of the number of input phage, a control plate containing the same phage suspension, but not bacterial cells with overlay, was used. The indicator strain for titer of χ phage was strain YK102. P1-mediated transductions were carried out as described before (8).

Isolation of motile χ phage insensitive mutants. The mutants that were motile but χ phage insensitive were isolated by the method of T. Icho (personal communication). The Fla⁻ mutants were streaked on a motility agar plate that contained 10^{10} plaque-forming units (PFU) of χ phage. After overnight incubation at 37°C, χ-insensitive motile clones formed swarms. They were cloned three times on L-agar plates and rechecked for χ insensitivity on motility agar plates containing χ phage. The *flaE694* allele was introduced into each of the mutants by transduction with P1 phage grown on strain MS694 (*flaE694*) *his*⁺ and by selection of *his*⁺ transductants.

Lambda *E. coli* hybrids. Hybrid lambda *E. coli* DNA molecules containing region I *fla* genes were constructed by using λgtλc DNA as the vehicle and fragments of DNA obtained by *Eco*RI endonuclease digestion of ColE1 *E. coli* DNA hybrids carrying region I flagellar genes by the procedure described previously (16). A description of λ*fla* containing region I flagellar genes has been reported (9). Hybrid λ lysates were tested for their ability to transduce specific flagellar genes by cross-streaking them on λ lysogenic strains of *E. coli* that carried specific gene mutations, as described before (18). The infection of UV-irradiated cells of strains 159 or 159 (λ) was performed exactly as described by Jaskunas et al. (6). [^{35}S]methionine with a specific activity of 320 Ci/mM (New England Nuclear) was used to label the proteins. The preparation of samples for electrophoresis and the autoradiography have been described (18).

Complement fixation assay. The antigenic character of polyhooks and hook subunits was measured by the complement fixation procedure of Wasserman and Levine (28). The antisera used were: Ra923 for measurement of polyhooks and Ra926 for measurement of hook subunits. Polyhooks were purified from strains with *flaE* mutations by the method of Kagawa et al. (7).

Gel electrophoresis. Urea (8 M)-polyacrylamide gel electrophoresis (pH 8.7) was performed as described before (2), except a slab-gel apparatus was used. Two-dimensional gel electrophoresis was that described by O'Farrell (13).

RESULTS

Motile χ phage-insensitive mutants. Flagellotropic phage χ attacks motile strains of *E. coli* (12). Examination of the course of events in infection showed that χ became attached to a flagellum by its tail fibers and then travelled down to the base of the flagellum where it injected its DNA (15). A rotating flagellum is a prerequisite for χ infection (21, 26). Mutants can be isolated that are resistant to χ phage infection and that still form flagella functional for motility and chemotaxis (5). An analysis of these kinds of mutants may reveal the nature of the interaction between flagella and χ phage. So far, the mutation sites in these mutant strains have been

mapped in or near the structural gene for flagellin (T. Icho and T. Iino, manuscript in preparation).

If the flagellar hook is part of the pathway in χ phage infection or a secondary attachment site for χ infection, there should be a hook subunit gene mutant that does not permit χ attachment, and therefore χ infection. Therefore, we attempted to isolate motile χ-insensitive mutants as revertants of region I Fla⁻ mutants.

Initially, the reversion frequency of Fla⁻ region I mutants to Fla⁺ was examined (Table 1). When the region I Fla⁻ strains were treated with ethyl methane sulfonate (EMS), the frequency of reversion was raised. EMS-treated Fla⁻ mutants were streaked on motility agar containing χ phage (10^9 PFU/ml). As shown in Table 1, motile χ-insensitive mutants were isolated only from a *flaK* amber mutant, strain YK2008. Two strains from each complementation group in region I were tested. χ-Sensitive revertants could be isolated from all region I Fla⁻ strains, but we could not isolate χ-insensitive mutants from the other Fla⁻ mutants. One χ-sensitive motile mutant of YK2008 (strain YK3001) and four χ-insensitive (strains YK3010 through YK3013) motile revertants of strain YK2008 were saved and used for further examination. The location of the mutation sites in the χ-insensitive mutants was analyzed by P1-mediated transduction. P1 lysates grown on these mutants were used to transduce *fla* genes into representative Fla⁻ mutants on motility agar. The recombinant clones (swarm formers) were checked for their χ phage sensitivity. The data are shown in Table 2. The mutational site conferring χ insensitivity was transduced most frequently with the *flaK* gene, and the insensitive alteration may be in or near the *flaK* gene.

Antigenicity of hooks from χ-insensitive mutants. The antigenic specificity of the hooks isolated from the motile χ-insensitive mutants described above was examined with the complement fixation assay. To facilitate the recovery of large amounts of hook subunit protein, the *flaE694* mutation was introduced into each strain so that polyhooks would be produced (19). The mutation *flaE694* was introduced to strains YK102, YK3001, YK3010, YK3011, YK3012, and YK3013 with P1 phage, using *his*⁺ as the selec-

TABLE 1. *Reversion of region I Fla⁻ mutants*[a]

Strain	Gene defect	Mutagen	Cells examined	Fla⁺ clones	χ-Insensitive clones
YK2007	*flaV*	None	2.1×10^{10}	210	0
YK2008	*flaK*	None	2.1×10^{10}	313	0
YK2015	*flaL*	None	2.1×10^{10}	215	0
YK2017	*flaM*	None	2.1×10^{10}	113	0
YK2001	*flaS*	None	2.1×10^{10}	750	0
YK2002	*flaT*	None	2.1×10^{10}	517	0
YK2007	*flaV*	EMS	1.0×10^{9}	112	0
YK2008	*flaK*	EMS	1.0×10^{9}	131	51
YK2015	*flaL*	EMS	1.0×10^{9}	103	0
YK2017	*flaM*	EMS	1.0×10^{9}	110	0
YK2001	*flaS*	EMS	1.0×10^{9}	303	0
YK2002	*flaT*	EMS	1.0×10^{9}	278	0

[a] Reversion to Fla⁺ was examined on motility agar. Fla⁺ χ-insensitive clones were screened directly by plating the cells on motility plates containing χ phage (10^{10} PFU per motility plate).

TABLE 2. *Frequency of cotransduction of χ-insensitive character with region I flagellar genes*[a]

Recipient	Donor							
	YK3010		YK3011		YK3012		YK3013	
	χ^r/Fla⁺	Ratio	χ^r/Fla⁺	Ratio	χ^r/Fla⁺	Ratio	χ^s/Fla⁺	Ratio
YK2007 (FlaV⁻)	85/91	0.93	90/96	0.94	83/95	0.87	81/90	0.90
YK2008 (FlaK⁻)	87/87	1.00	93/93	1.00	96/96	1.00	96/96	1.00
YK2015 (FlaL⁻)	92/95	0.97	90/94	0.96	90/96	0.94	81/92	0.88
YK2017 (FlaM⁻)	71/90	0.79	80/93	0.96	75/90	0.83	80/96	0.85
YK2001 (FlaS⁻)	65/92	0.71	64/94	0.68	70/96	0.73	65/92	0.71
YK2002 (FlaT⁻)	61/95	0.64	58/92	0.63	59/95	0.62	60/91	0.66

[a] P1-mediated transductions were carried out as described in the text. Fla⁺ transductants were examined for χ phage sensitivity on motility plates containing χ phage (10^{10} PFU per plate).

tive marker. FlaE⁻ strain YK102 is designated YK3100, FlaE⁻ YK3001 is strain YK3101, FlaE⁻ YK3010 is strain YK3110, FlaE⁻ YK3011 is strain YK3111, FlaE⁻ YK3012 is strain YK3112, and FlaE⁻ YK3013 is strain YK3113. The polyhooks were sheared from whole bacteria and concentrated by centrifugation (see above). Polyhooks from wild-type and χ-insensitive mutants were examined with the electron microscope and found to be identical in gross appearance. The results of complement fixation analysis are shown in Fig. 2. Strains YK3100 (parent strain), YK3101 (χ-sensitive motile revertant), YK3110 and YK3111 (χ-insensitive mutant) made polyhooks that could react with antibody against wild-type polyhook. Polyhooks from strains YK3110 and YK3111, however, showed considerably less reactivity. Polyhooks from two of the motile χ-insensitive mutants, strains YK3112 and YK3113, did not show complement-fixing activity. Furthermore, the antibody against the polyhook monomer did not react with the polyhook subunit from the χ-insensitive mutants YK3112 and YK3113.

Gel electrophoresis of polyhook protein from the revertants of FlaK⁻ mutants. Hook protein isolated from polyhook structure of the χ-insensitive revertants of strain YK2008 was compared with that of the parent strain (YK3100) by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, urea-polyacrylamide gel electrophoresis, and two-dimensional gel electrophoresis. On an 8 M urea gel the hook monomer for strains YK3112 and YK3113 electrophoresed much more rapidly than the hook monomer from the wild-type or χ-sensitive mutant (Fig. 3). In comparison with the wild-type monomer, the hook monomer from strain YK3112 electrophoresed to a more alkaline pH in the isoelectric focusing dimension of the two-dimensional gel of O'Farrell (Fig. 4). On one-dimensional SDS-gels, the hook monomer from the χ-insensitive mutants (YK3112 and YK3113) electrophoresed more rapidly. The hook subunit monomer from strain YK3112 was approximately 40,000 molecular weight and that from strain YK3113 was 38,000 molecular weight (data not shown). Thus, mutation in the *flaK* gene (reversion to Fla⁺ and χ insensitivity) resulted in alteration in the structure of the hook monomer.

FlaK gene on λ *E. coli* hybrids. Figure 1 shows the location of region I flagellar genes on the *E. coli* genome (9). A phage lambda carrying region I *fla* genes was isolated by using molecular cloning techniques. The hybrid lambda was constructed using λgtλc DNA as the vehicle and fragments of DNA obtained by *Eco*RI endonuclease digestion of ColE1 *E. coli* hybrid plasmid

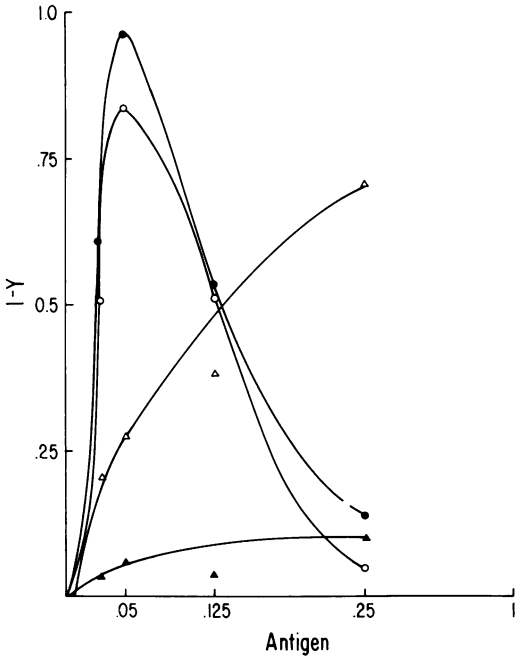

FIG. 2. *Antigenicity of polyhook preparations measured by complement fixation. Complement fixation was performed as described in the text. Polyhook antiserum Ra923 was used at a dilution of 1/7,000. I-Y is "complement fixation" as defined by Wasserman and Levine (28). The amount of polyhook protein added to the reaction is in micrograms. Polyhook proteins used are from strains YK3100* (○), *YK3101* (●), *YK3110* (△), *and YK3113* (▲).

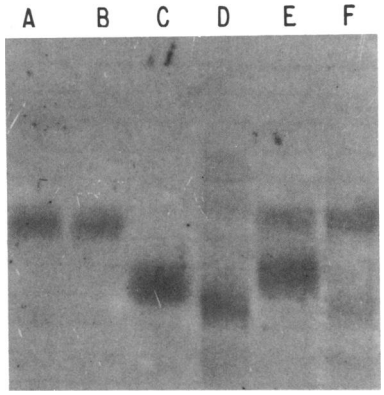

FIG. 3. *Urea-polyacrylamide gel electrophoresis. Approximately 3 μg of polyhook protein from each mutant strain was added to each well of an 8 M urea (pH 8.7) polyacrylamide slab gel: (A) polyhook protein from strain YK3100; (B) from strain YK3101; (C) from strain YK3112; (D) from strain YK3113; (E) from strains YK3100 and YK3112; (F) from strains YK3100 and YK3113.*

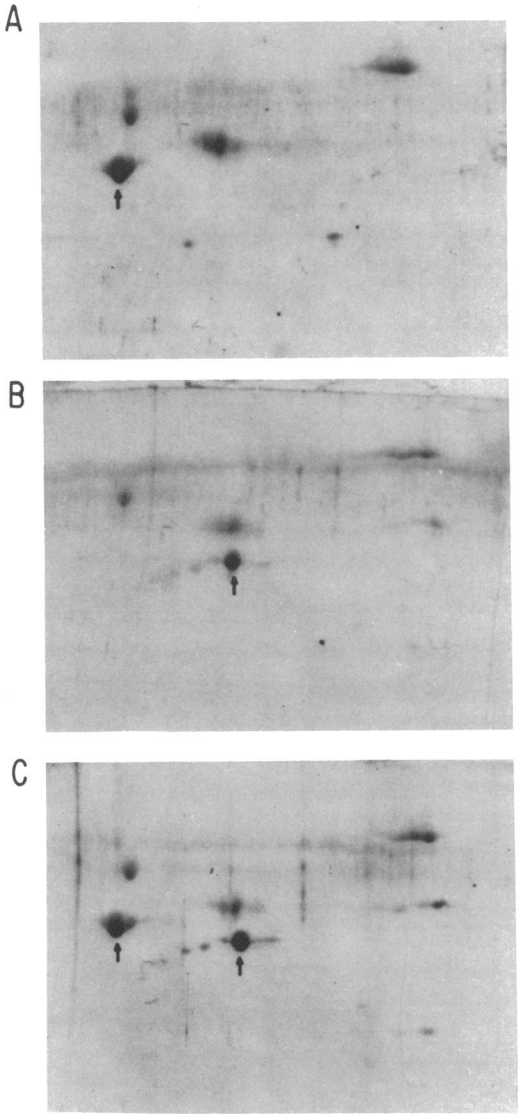

FIG. 4. *Two-dimensional gel electrophoresis of hook subunit proteins from mutant strains. Two-dimensional electrophoresis was performed by the method of O'Farrell (13). Gels are: A, hook protein from strain YK3100; B, from strain YK3112; C, from strains YK3100 and YK3112. A 3-μg portion of hook protein from each strain was subjected to electrophoresis with marker proteins in each gel. The marker proteins used were: bovine serum albumin (molecular weight 68,000), E. coli flagellin (molecular weight 54,000), and ovalbumin (molecular weight 43,000). Arrows indicate the position of hook proteins. The pH gradient runs from left to right starting at pH 3.0 and going to pH 8.5. SDS electrophoresis is from top to bottom.*

pLC36-11 carrying region I flagellar genes as the passenger (1). This hybrid, λ*fla*6 carried *flaM*, *flaL*, *flaK*, and the promotor distal part of *flaV*, as determined by genetic complementation and recombination tests. The hybrid did not contain the entire region I *fla* gene operon. A hybrid λ*fla* containing the entire set of region I *fla* genes has been isolated and the details of its construction have been reported (9). Hybrid λ*fla*69 had genes *flaV*, *flaK*, *flaL*, and *flaM*, whereas hybrid λ*fla*691 had the genes *flaV*, *flaK*, *flaL*, *flaM*, *flaS*, and *flaT*. We used the pyrophosphate shock method (14) for the isolation of deletion mutants of λ*fla*691. Various lambda *fla* deletion mutants were characterized genetically by transductional crosses and were also used to program protein synthesis in UV-irradiated cells. Figure 5 shows that the *flaK* genetic activity correlated with the capacity to direct the synthesis of a polypeptide that had an apparent molecular weight of 42,000. The 42,000-molecular weight polypeptide was programmed by λ*fla*691Δ16 (*flaV flaK*) and λ*fla*691Δ17 (*flaV flaK flaL*), but not by λ*fla*69Δ1 (*flaV*). When strain MS6013 (a *flaI* mutant of 159 [λ]) (23) was used as host, no spot corresponding to molecular weight 42,000 was detected. The material corresponding to molecular weight 42,000 programmed by λ*fla* co-electrophoresed with the hook subunit protein obtained from hook basal body structures (4) on both one- and two-dimensional gels.

Sensitivity of Fla⁻ mutants to χ phage. In *Salmonella*, H1⁻ and H2⁻ mutants that possess only the hook-basal body complex showed sensitivity to the phage χ (27). Therefore, if partial flagellar organelles exist in the cell envelopes of Fla⁻ mutants, these mutants may be capable of supporting phage infection. Strains with region I *fla* mutation were tested for the capacity to support χ infection. Since χ phage did not form plaques on these mutants, indirect test of phage growth was used (see above). Table 3 shows that FlaK⁻ mutants consistently yielded more χ phage than other strains with region I *fla* mutations. This varied from approximately 3 to 100 times higher yields at an input of 1×10^6 PFU per plate. When mutants with deletions of region I *fla* genes were used as hosts, no significant increase in χ titer was observed. Thus, FlaK⁻ mutants were better hosts for χ infection than other mutants with region I *fla* defects.

DISCUSSION

Several lines of evidence support the conclusion that the *flaK* gene is the structural gene for the hook subunit protein. Like other Fla⁻ mutants an FlaK⁻ mutant could revert to the functional Fla⁺ phenotype, but in contrast to revertants of other Fla⁻ mutant groups, some revertants of an FlaK⁻ mutant were insensitive to infection with the flagellotropic phage χ. By

analogy with a class of Fla^+ χ-insensitive mutants in *Salmonella*, which result from mutation in the structural gene for flagellin (5), the genetic change in the $FlaK^+$ revertants must be in a flagellar component on the route of χ infection.

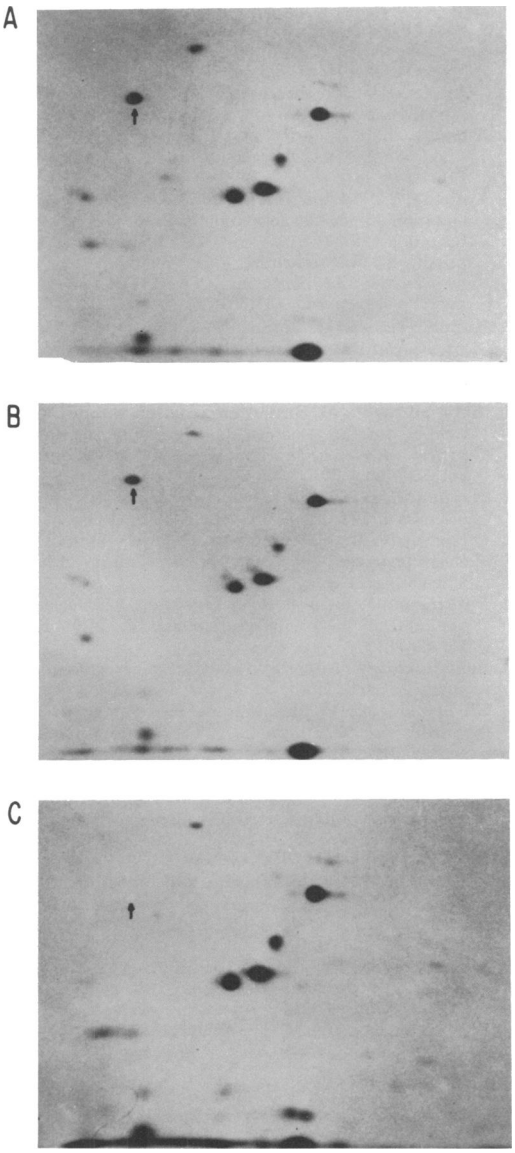

FIG. 5. *Programming the synthesis of hook protein in UV-irradiated cells. Infection of UV-irradiated cells with λ E. coli hybrids was performed as described in the text. The labeled proteins were separated by two-dimensional gel electrophoresis as in Fig. 4. The λ-E. coli hybrids used were: (A) λfla691Δ17 (flaV⁺K⁺L⁺). (B) λ691Δ16 (flaV⁺K⁺); (C) λfla69Δ1 (flaV⁺). The arrows indicate the position of authentic hook subunit protein from purified hookbasal body structures (4). Autoradiogram was exposed for 1 week.*

TABLE 3. *χ Phage growth on region I Fla⁻ mutants*[a]

Strain	Phenotype	χ Phage yield (PFU/ml)	
		Input PFU (1×10^3)	Input PFU (1×10^6)
YK102	Fla^+	3.2×10^8	6.1×10^9
YK2007	$FlaV^-$	2.1×10^3	3.1×10^7
YK2022	$FlaV^-$	1.3×10^3	5.2×10^7
YK2003	$FlaK^-$	4.8×10^3	2.2×10^8
YK2008	$FlaK^-$	6.0×10^3	2.0×10^8
YK2015	$FlaL^-$	2.3×10^3	5.1×10^7
YK2040	$FlaL^-$	3.1×10^3	7.1×10^7
YK2017	$FlaM^-$	2.4×10^3	3.1×10^7
YK2038	$FlaM^-$	1.9×10^3	5.0×10^7
YK2001	$FlaS^-$	9.8×10^2	2.1×10^6
YK2002	$FlaT^-$	9.8×10^2	6.8×10^6
YK2253	Fla (VKLMST)⁻	8.8×10^2	9.3×10^5
YK2258	Fla (VKLM)⁻	9.9×10^2	9.0×10^5

[a] χ Phage was grown on each mutant strain as described in the text.

The most reasonable expectation would be that the hook subunit was altered and *flaK* was the gene that specified the hook subunit. We sought to make a comparison between the hook protein subunits from the χ-insensitive mutants and the protein from the χ-sensitive mutant or the wild-type strain. To facilitate this comparison, the polyhook phenotype was introduced into all the relevant strains by transduction of the *flaE694* mutation. With large amounts of hook protein now available, it was apparent that the polyhooks (or hook monomers) from two χ-insensitive revertants did not cross-react with antisera to wild-type polyhooks or to wild-type hook subunits, whereas the polyhooks (or hook monomers) from the χ-sensitive mutants did. Furthermore, the hook subunit proteins from the χ-insensitive revertants differed substantially in their electrophoretic mobility in urea acrylamide gels, SDS-acrylamide gels, and in the isoelectric focusing dimension of two-dimensional polyacrylamide gels. Thus, the genetic change in the *flaK* gene, which gave rise to Fla^+ χ-insensitive cells, also resulted in alteration in the properties of the hook subunit protein. The mutation conferring χ insensitivity was transduced with the *flaK* gene, so it is unlikely that the alteration in the hook subunit protein resulted from a suppressor mutation in a gene other than *flaK*. The hook proteins from two χ-insensitive mutants had a lower molecular weight than did the wild-type polypeptide. Several explanations could account for the occurrence of functional, yet abbreviated, *flaK* gene products. For example, the original defect in $FlaK^-$ strain YK2008 was an amber mutation, i.e., it was suppressed by ϕ80SuIII phage, and reversion to Fla^+ may have resulted from a base deletion (frame shift mutation), which suppressed the original amber mutations but did not permit reading of the

entire *flaK* gene. If this were the case, the missing amino acid sequences would be located at the C-terminal end of the hook polypeptide. The polyhooks composed of the abbreviated hook subunit proteins from the χ-insensitive mutants did not react with antiserum to wild-type polyhooks. If the amino acid sequences missing from the variant proteins were located at the C-terminal end, we would expect the antigenic sites for the hook protein to be determined by the C-terminal sequences of the hook protein. By a similar argument, the amino acid sequence in the hook protein, which is important for χ phage interaction, may be on the C-terminal portion of the hook protein molecule.

Hybrid λ*fla* containing the flagellar genes in region I programmed a 42,000-molecular weight polypeptide identical to the hook subunit progein on SDS-polyacrylamide gel electrophoresis. The synthesis of this polypeptide was under *flaI* gene control. Deletion of hybrid λ*fla*, which removed the *flaK* genetic activity, also removed the capacity of the λ to program the synthesis of the 42,000-molecular weight polypeptide. The capacity to synthesize the 42,000-molecular weight polypeptide was always correlated with *flaK* genetic activity. Thus, the *flaK* gene product by this independent analysis was the hook protein subunit.

The χ phage sensitivity that FlaK$^-$ mutants showed relative to other region I Fla$^-$ mutants is interesting in light of the function of the *flaK* gene. The differential sensitivity to the flagellotropic phage might be explained by the presence of a basal structure in FlaK$^-$ mutants, which acts as receptor for the χ phage. Mutants in the other components of the flagellar base may still be able to form a structure that would allow some degree of phage attachment and injection. The relative sensitivity of these mutants could define the nature of such structures. As identification of genes for other components of the flagellar apparatus is accomplished, it may be possible to further explore the anatomy of the flagellar rotor.

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LITERATURE CITED

1. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic Col E1 hybrid plasmids representative of the entire *Escherichia coli* genome. Cell **9**:91-99.
2. **Davis, B.** 1964. Disc electrophoresis. Ann. N.Y. Acad. Sci. **121**:404-427.
3. **DePamphilis, M. L., and J. Adler.** 1971. Fine structure and isolation of the hook-basal body complex of flagella from *Escherichia coli* and *Bacillus subtilis*. J. Bacteriol. **105**:384-395.
4. **Hilmen, M., and M. I. Simon.** 1976. Motility and the structure of bacterial flagella, p. 35-45. *In* R. Goddson, T. Pollard, and J. Rosebaum (ed.), Cold Spring Harbor Conference on Cell Proliferation and Motility, vol. 3. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
5. **Iino, T.** 1964. Genetical studies of *Salmonella* flagella. Jpn. J. Genet. **39**:313-335 (In Japanese).
6. **Jaskunas, S. R., L. Lindahl, M. Nomura, and R. R. Burgess.** 1975. Identification of two copies of the gene for the elongation factor EF-Tu in *E. coli*. Nature (London) **257**:458-462.
7. **Kagawa, H., S. Asakura, and T. Iino.** 1973. Serological study of bacterial flagellar hooks. J. Bacteriol. **113**:1474-1481.
8. **Komeda, Y., T. Icho, and T. Iino.** 1977. Effects of *galU* mutation on flagellar formation in *Escherichia coli*. J. Bacteriol. **129**:908-915.
9. **Komeda, Y., M. Silverman, and M. Simon.** 1977. Genetic analysis of *Escherichia coli* K-12 region I flagellar mutants J. Bacteriol. **131**:801-808.
10. **Kondoh, H., and H. Hotani.** 1974. Flagellin from *Escherichia coli* K12: polymerization and molecular weight in comparison with *Salmonella* flagellins. Biochim. Biophys. Acta **336**:119-139.
11. **Matsumura, P., M. Silverman, and M. Simon.** 1977. Cloning and expression of the flagellar hook gene by hybrid plasmids in minicells. Nature (London) **265**:758-760.
12. **Meynell, E. W.** 1961. A phage, φχ, which attacks motile bacteria. J. Gen. Microbiol. **25**:253-290.
13. **O'Farrell, P. H.** 1975. High-resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007-4021.
14. **Parkinson, J. S., and R. J. Huskey.** 1971. Deletion mutants of bacteriophage lambda. J. Mol. Biol. **56**:369-384.
15. **Schade, S. Z., J. Adler, and H. Ris.** 1967. How bacteriophage χ attacks motile bacteria. J. Virol. **1**:599-609.
16. **Silverman, M., P. Matsumura, R. Draper, S. Edwards, and M. Simon.** 1976. Expression of flagellar genes carried by bacteriophage lambda. Nature (London) **261**:248-250.
17. **Silverman, M., P. Matsumura, M. Hilmen, and M. Simon.** 1977. Characterization of lambda *Escherichia coli* hybrids carrying chemotaxis genes. J. Bacteriol. **130**:877-887.
18. **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.
19. **Silverman, M. R., and M. I. Simon.** 1972. Flagellar assembly mutants in *Escherichia coli*. J. Bacteriol. **112**:986-993.
20. **Silverman, M., and M. Simon.** 1973. Genetic analysis of flagellar mutants in *Escherichia coli*. J. Bacteriol. **113**:105-113.
21. **Silverman, M., and M. Simon.** 1974. Flagellar rotation and the mechanism of bacterial motility. Nature (London) New Biol. **249**:73-74.
22. **Silverman, M., and M. Simon.** 1974. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J. Bacteriol. **120**:1196-1203.
23. **Silverman, M., and M. Simon.** 1976. Operon controlling motility and chemotaxis in *E. coli*. Nature (London) **264**:577-580.
24. **Silverman, M., and M. Simon.** 1977. Identification of polypeptides necessary for chemotaxis in *Escherichia coli*. J. Bacteriol. **130**:1317-1325.
25. **Silverman, M., and M. Simon.** 1977. Bacterial flagella.

Annu. Rev. Microbiol. **31**:397–419.

26. **Thipayathasana, P., and R. Valentine.** 1974. The requirement for energy transducing ATPase for anaerobic motility in *Escherichia coli*. Biochim. Biophys. Acta **347**:464–468.
27. **Yamaguchi, S., H. Fujita, T. Kuroiwa, and T. Iino.** 1977. Sensitivity of non-flagellate *Salmonella* mutants to the flagellotropic bacteriophage χ. J. Gen. Microbiol. **99**:209–214.
28. **Wasserman, E., and L. Levine.** 1961. Quantitative microcomplement fixation and its use in the study of antigen structure. J. Immunol. **87**:290–296.