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Identification and Characterization of the Products of Six Region III Flagellar Genes (*flaAII.3* through *flaQII*) of *Salmonella typhimurium*

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A portion of flagellar region III of the *Salmonella typhimurium* genome has been cloned and shown to contain six genes: *flaAII.3*, *flaAIII*, *flaS*, *flaR*, *flaQI*, and *flaQII*. Of these, all but *flaQI* were known to exist from mutant studies; the former *flaQ* has been renamed *flaQII*. The genes were shown by minicell analysis to encode proteins with apparent molecular masses of 28, 48, 15, 46, 17, and 37 kilodaltons, respectively. The presence of a flagellar-gene-specific promoter in the vicinity of *flaQI* was established by testing expression of the plasmid-encoded tetracycline resistance gene in artificial constructions. In minicell preparations, the *flaAII.3* and *flaR* products were found principally in the cytoplasmic fraction; the rest were found principally in the membrane fraction. A comparison between the homologous genes of *S. typhimurium* and *Escherichia coli* confirmed that their genomic organizations were similar and that their products had similar molecular masses and isoelectric points.

Many bacteria have organelles called flagella that rotate by using the transmembrane proton motive force as their energy source. Because of the helical shape of the external flagellar filament, rotation provides thrust that results in cell locomotion. Flagellar rotation can occur in either the counterclockwise or the clockwise sense, with control of the two senses enabling the selective locomotion called taxis (for reviews, see references 21 and 22).

Flagellar formation and function in *Salmonella typhimurium* involves about 40 genes clustered in three regions (I, II, and III) of the genome. Genes that are essential for the formation of the bacterial flagellum are called *fla* genes. Others that are needed only for its rotation and for switching of its sense of rotation are called *mot* and *che* genes, respectively. Region I contains many structural genes for the filament hook-basal body complex of the flagellum; region II contains a few *fla* genes, two *mot* genes, and several chemotaxis genes; region III, the largest and least understood, contains at least 16 genes, all of which are involved in flagellar assembly.

The present study concerns region III genes (Table 1). Among these are a few structural ones for the filament hook-basal body complex: *H1* is one of two genes coding for the filament protein, flagellin (12); *flaV* codes for a hook-associated protein (9); *flaAII.1* is the structural gene for a 65-kilodalton (kDa) protein (1; M. Homma and R. M. Macnab, unpublished data) that constitutes the M ring of the basal body (7). There are also three genes whose products control the switching properties of the flagellar motor, namely, *flaAII.2*, *flaQ*, and *flaN* (34, 35). The product of one gene, *flaR*, plays a regulatory role, controlling the hook length during flagellar assembly (26, 30). For most of the rest of the region III genes, nothing is known of their roles except that no recognizable flagellar structure occurs in their absence.

Even when no detailed knowledge concerning the role of a given gene and its product is available from mutant phenotype or other information, it is valuable to know how that gene fits into the genomic organization, what the molecular mass and isoelectric point of its product are, and where the product is located within the cell. We have recently reported such information for the *flaFV* through *flaFIX* genes of region I of *S. typhimurium* (10). Here we report on six region III genes and compare their properties with those of *Escherichia coli*.

MATERIALS AND METHODS

Bacterial strains and plasmids. *S. typhimurium* LT2 (11) was used for preparation of chromosomal DNA. *E. coli* strains used are listed in Table 2. Plasmids used are described in Results.

Media and enzymes. Media and enzymes have been described elsewhere (9). Chemicals and enzymes were obtained from standard commercial sources.

Manipulation of DNA. Conventional recombinant DNA techniques were performed as described by Maniatis et al. (23).

Cloning of *fla* genes of *S. typhimurium*. *fla* genes were cloned as described elsewhere (9).

Minicell analysis. Proteins programmed by plasmids were analyzed by the minicell method described elsewhere (9), using the minicell-producing strain UH869.

Fractionation of minicell membrane, cytoplasm, and periplasm. Fractionation was performed as described in method 2 of reference 8.

Electrophoresis, staining, and fluorography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional gel electrophoresis were performed as described elsewhere (1). Fluorography was performed by the acetic acid-2,5-diphenyloxazole method as described elsewhere (8).

RESULTS

Cloning of region III genes of *S. typhimurium*. *S. typhimurium* chromosomal DNA and plasmid pBR322 were digested by *Eco*RI and ligated. The pool of recombinant plasmids was introduced into *E. coli* MHE103, a mutant defective in $flaE_E$,

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TABLE 1. Correspondence between flagellar region III genes of *S. typhimurium* and *E. coli*[a]

S. typhimurium gene	*E. coli* gene
flaL	$flaD_E$
nml	
H1	hag_E
flaV	$flbC_E$
flaAI	$flaN_E$
flaAII.1	$flaBI_E$
flaAII.2	$flaBII_E$
flaAII.3[b]	$flaBIII_E$[b]
flaAIII[b]	$flaC_E$[b]
flaS[b]	$flaO_E$[b]
flaR[b]	$flaE_E$[b]
flaQI[b]	$flaAI_E$[b]
flaQII[b]	$flaAII_E$[b]
flaN	$motD_E$
flaP	$flbD_E$
flaB	$flaR_E$
flaD	$flaQ_E$
flaX	$flaP_E$

[a] Genes are presented in genome order. The correspondences between homologous genes of *S. typhimurium* and *E. coli* are in most cases clearly established; the status of $motD_E$ and $flbD_E$ has been uncertain (25), but recent data indicate that they are distinct genes and occur in the order shown (P. Matsumura, personal communication). *nml* codes for a protein that methylatescertain lysine residues of flagellin (16).

[b] These genes are the subject of the present study; in the case of *E. coli* genes, they have also been the subject of previous studies (3, 17).

which is homologous to *flaR* of *S. typhimurium* (*E. coli* genes are indicated by a subscript E; see Table 1 for the correspondences between the region III genes of *S. typhimurium* and *E. coli*). From transformants that complemented MHE103 and were resistant to tetracycline and ampicillin, two plasmids, PMH1 and pMH2, were recovered. They were shown to contain the same 5.3-kilobase chromosomal *Eco*RI-*Eco*RI fragment in opposite orientations. Both plasmids complemented not only MHE103 but also $flaBIII_E$, $flaC_E$, $flaO_E$, and $flaAII_E$ mutants, demonstrating that they contained the homologous *S. typhimurium* genes: *flaAII.3*, *flaAIII*, *flaS*, *flaR*, and *flaQII* (formerly *flaQ*; see below). They failed to complement a $flaBII_E$ and a $motD_E$ mutant, indicating that genes upstream of *flaAII.3* (*flaAII.2*, etc.) and downstream of *flaQ* (*flaN*, etc.) were not present in intact form.

Various deletion derivatives of pMH1 or pMH2 were constructed and tested for the ability to complement nonflagellate *E. coli* mutants. The results (Fig. 1) indicated that the *flaAII.3*, *flaAIII*, *flaS*, *flaR*, and *flaQII* genes were located in restriction fragments RI(1)-M(1), RI(1)-S, P(2)-H(1), S-C, and H(2)-RI(2), respectively (see the legend to Fig. 1 for fragment designations).

Identification of the *flaAII.3*, *flaAIII*, *flaS*, *flaR*, and *flaQII* gene products. The radiolabeled proteins synthesized in a minicell strain under the direction of various plasmids (Fig. 2; other examples not shown) were compared with the complementation data, enabling us to identify the products of the cloned genes. Thus, for example, pMH2, pMH21, pMH22, pMH26, and pMH1 resulted in the synthesis of a protein with a molecular mass of 48 kDa (as estimated by mobility in SDS-PAGE), while pMH23, pMH24, pMH11, pMH12, pMH261, pMH111, and pMH112 (the latter three not shown in Fig. 2) did not, indicating that this protein was the *flaAIII* gene product; similarly, the data indicated that proteins with apparent molecular masses of 15, 28, 37, and 46 kDa were the *flaS*, *flaAII.3*, *flaQII*, and *flaR* products, respectively. The *flaQII* and, to a lesser extent, the *flaAII.3* products were obscured by plasmid-encoded proteins in one-dimensional electrophoretic gels but could readily be detected in two-dimensional gels (Fig. 3; other examples not shown) in a manner consistent with the complementation analysis. (Subsequent DNA sequence analysis has revealed that the *Eco*RI site at the insert-pBR322 junction of pMH2 lies just before the 3′ end of the *flaQII* gene and that, as a consequence, the 37-kDa protein, though functional, differs slightly from the true *flaQII* gene product, lacking eight C-terminal FlaQII residues and containing, instead, three residues encoded by pBR322 DNA [M. Kihara, M. Homma, and R. M. Macnab, unpublished data]. The protein will therefore be referred to as FlaQII′ to indicate this fact. The properties of the intact *flaQII* product, together with those of other region III genes downstream, are currently under study.)

The isoelectric points of the FlaAII.3, FlaAIII, FlaR, and FlaQII′ proteins were all in the acid-to-neutral range, while that of FlaS was moderately basic (Fig. 3). FlaAIII and FlaS had a punctate, streaky appearance. This phenomenon is quite common, especially for proteins with basic isoelectric points (e.g., the FlaFVIII and FlaFIX proteins; 10); its origin is uncertain.

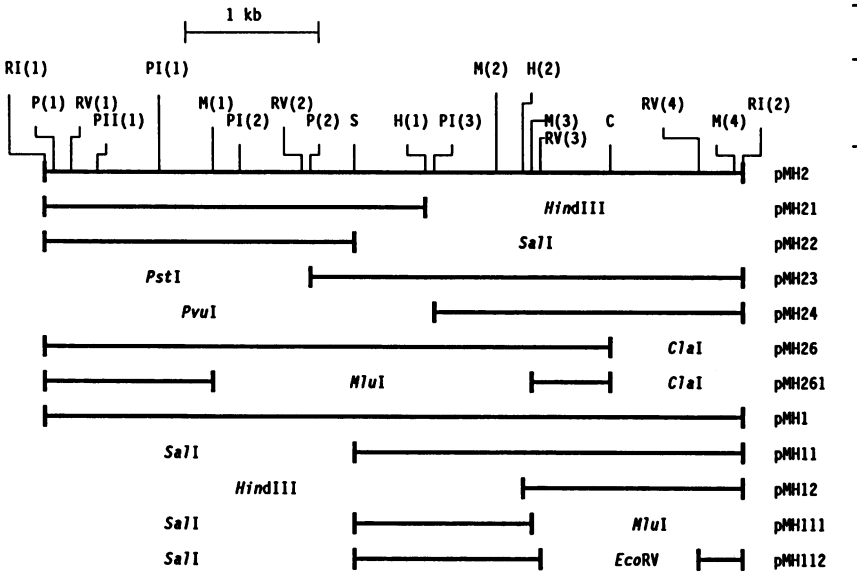
All plasmids that directed synthesis of the 46-kDa *flaR* product were also found to produce a 45.5-kDa protein (Fig. 2) with a similar isoelectric point (Fig. 3). It seems likely that the 45.5-kDa protein is related to the *flaR* product, but we were unable to determine whether it is simply a degradation product or a biologically significant processed form.

Identification of a second *flaQ* product. Plasmids containing DNA between the H(2) and RI(2) restriction sites (Fig. 1) of pMH2 were found to direct synthesis of a quite basic 17-kDa protein (Fig. 2 and 3), as well as the 37-kDa protein described above. This 17-kDa protein was also detected with plasmid pMH26, which has all chromosomal DNA from the *Cla*I site to the end of the insert deleted and which neither synthesized the 37-kDa protein nor complemented a $flaAII_E$ mutant. Thus, the gene encoding the 17-kDa protein was

TABLE 2. *E. coli* bacterial strains

Strain	Relevant characteristics[a]	Reference
EKK9	F⁻ *thr leu met hsdR hsdM supE*	20
MHE103	$flaE_E$ of EKK9	This study
RP437	F⁻ *thi thr leu strA eda*	25
MS1350	F⁻ *his thy argE uvrC galU sup*⁺ Str^r Fla⁺ *hag-207*	28
W3623H	F⁻ *his trpA gal* Str^r	15
YK410	F⁻ *araD139 lacU169 rpsL thi pyrC46 gyrA thyA his*	13
RP4501	$flaBII_E$ (= *scyB10*) *supD* of RP437	25
MS131	$flaBIII_E$ of MS1350	3
YK4160	$flaA_E$ of YK410	13
RP4187	$flaA_E75$(Am) of W3623H	25
YK4105	$flaE_E$ of YK410	13
MS694	$flaE_E$ of MS1350	28
YK4112	$flaO_E$ of YK410	13
YK4178	$flaC_E$ of YK410	3
RP4189	$flaC_E87$(Am) of W3623H	25
YK4117	$motD_E$ of YK410	13
YK4148	$flaM_E$ of YK410	13
YK4136	$flaI_E$ of YK410	13
YK4116	$flbB_E$ of YK410	13
UH869	F⁻ *minA minB mgl*(?) *rpsL recA*	9

[a] Flagellar gene symbols have a subscript E to indicate that they refer to *E. coli*; the symbols for homologous *S. typhimurium* genes are not in general the same.



	Motility				
	$flaBIII_E$ (*flaAII.3*)	$flaC_E$ (*flaAIII*)	$flaO_E$ (*flaS*)	$flaE_E$ (*flaR*)	$flaAII_E$ (*flaQII*)
pMH2	+	+	+	+	+
pMH21	+	+	+	-	-
pMH22	+	+	-	-	-
pMH23	-	-	+	+	+
pMH24	-	-	-	-	+
pMH26	+	+	+	+	-
pMH261	+	-	-	-	-
pMH1	+	+	+	+	+
pMH11	-	-	-	+	+
pMH12	-	-	-	-	+
pMH111	-	-	-	-	-
pMH112	-	-	-	-	-

FIG. 1. Linearized physical maps of plasmids carrying *S. typhimurium* DNA (continuous horizontal line) in the vicinity of flagellar region III genes *flaAII.3* through *flaQII* (cf. Table 1) and the ability of these plasmids to confer motility on *E. coli* mutants. *E. coli* genes are indicated by a subscript E, and the homologous *S. typhimurium* genes are shown below in parentheses. Single colonies of ampicillin-resistant transformants were inoculated on a semisolid agar plate and incubated at 37°C for 10 h. On the basis of the complementation tests, the *S. typhimurium* genes are inferred to fall within the deletion endpoints given in the text. Restriction endonuclease sites are labeled as follows: C, *Cla*I; RI, *Eco*RI; RV, *Eco*RV; H, *Hin*dIII; M, *Mlu*I; P, *Pst*I; PI, *Pvu*I; PII, *Pvu*II; S, *Sal*I. Multiple occurrences are indicated by the numbers in parentheses.

inferred to be upstream of the gene—until now known as *flaQ*—that encodes the 37-kDa protein. This inference is consistent with the finding of an additional gene in this region by DNA sequence analysis (M. Kihara, M. Homma, and R. M. Macnab, unpublished data). We have therefore given the name *flaQI* to the gene coding for the 17-kDa protein and renamed, as *flaQII*, the gene coding for the 37-kDa protein. No mutant defective in *flaQI* has yet been described.

A similar situation has previously been shown to apply in *E. coli*, both by sequence analysis and product detection (5, 17); the genes for the two *E. coli* proteins (molecular masses, 17 and 37 kDa) are $flaAI_E$ and $flaAII_E$, respectively.

A flagellar-gene-specific promoter for the *flaQI* operon. The *E. coli* gene $flaAI_E$ is the first of an operon (17) that includes $flaAII_E$, $motD_E$, $flbD_E$, $flaR_E$, $flaQ_E$, and $flaP_E$ (25, 29). Since the flagellar genetics of *E. coli* and *S. typhimurium* are very similar, it was likely that the cloned region III DNA of *S. typhimurium* in pMH2 contained the promoter for an operon whose leading gene was *flaQI*. This operon should be under flagellar-gene-specific control by the master operon (containing *flaK* and *flaE* in *S. typhimurium* and $flbB_E$ and $flaI_E$ in *E. coli*) of the flagellar regulon (2, 13, 14, 19) and should be at an intermediate level (group 3) in the overall hierarchy of regulation described by Komeda (14).

We therefore examined what DNA was needed for flagellar-gene-specific expression, in various host backgrounds, of the plasmid-encoded tetracycline resistance gene (which lies downstream of the *S. typhimurium* insert) (Fig. 4). Plasmid pMH2, with the natural tetracycline resistance promoter intact, provided resistance even to $flbB_E$ or $flaI_E$ mutants. Plasmid pMH26, a *Cla*I-*Cla*I deletion derivative of pMH2 in which the natural promoter had been destroyed, conveyed tetracycline resistance to a wild-type strain or a mutant defective in a group 3 gene ($flaM_E$) but not to $flbB_E$ or $flaI_E$ mutants; the same results were obtained with pMH261, a *Mlu*I-*Mlu*I deletion derivative of pMH26. Plasmid pMH21, a pMH2 derivative with a somewhat larger deletion, did not provide tetracycline resistance in any host. These results show (Fig. 4) that within the ca. 0.7-kilobase M(2)-C restriction fragment there is a promoter that is active only when the flagellar regulon is activated. As predicted from the scheme of Komeda, it does not appear to require expression of other group 3 genes such as $flaM_E$.

Localization of gene products to subcellular fractions. Radiolabeled minicells containing pMH1 were fractionated as described previously (method 2 in reference 8). Minicell spheroplasts, obtained by treatment with lysozyme, were collected by centrifugation; the supernatant was defined as the periplasmic fraction. The spheroplasts were then sonicated and centrifuged at high speed; the supernatant and pellet from this were defined as the cytoplasmic and membrane (both cell and outer) fractions, respectively. The gene products were found to be primarily in the following fractions (Fig. 5): cytoplasm, FlaAII.3 and FlaR; periplasm, β-lactamase; membrane, pre-β-lactamase, FlaAIII, FlaS, FlaQI, and FlaQII′. The 45.5-kDa protein that appears to be related to FlaR (see above) fractionated differently from it, appearing most strongly in the periplasm; the reason for this, like the origin of this protein, is unclear.

Comparison with the homologous gene products of *E. coli*. The *E. coli* genes homologous to the *S. typhimurium* genes being studied here have already been cloned, and their products have been isolated (3, 17). We wished to compare directly the gene products of the two species.

The $flaBIII_E$, $flaC_E$, $flaO_E$, $flaE_E$, $flaAI_E$, and $flaAII_E$ genes were previously cloned in plasmid pDB7, in which the *lacUV5* promoter lies just upstream of the $flaBIII_E$ gene (3). After the restriction sites and complementation behavior of pDB7 and the deletion derivative plasmids we had prepared from it (Fig. 6) were confirmed, the gene products were

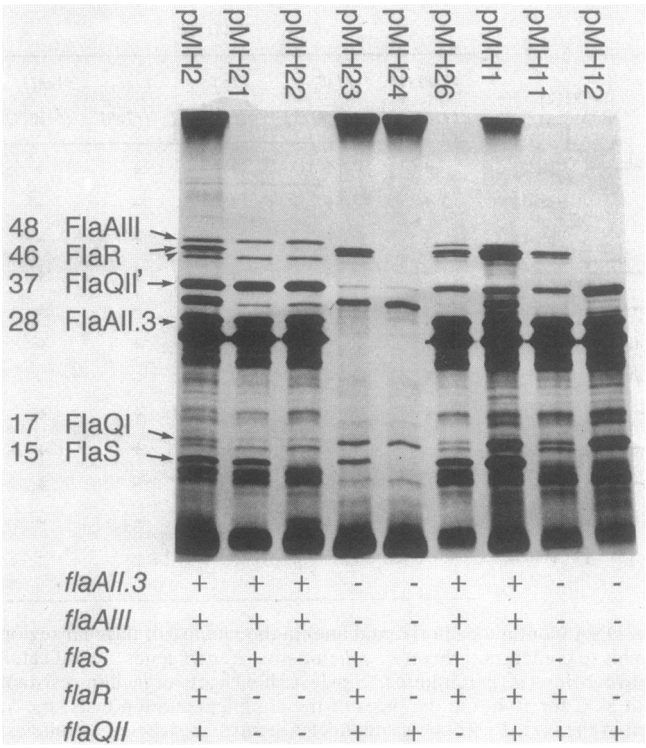


FIG. 2. Identification of the products of the *flaAII.3*, *flaAIII*, *flaS*, *flaR*, *flaQI*, and *flaQII* genes of *S. typhimurium*. Proteins were radiolabeled in minicells containing the plasmids shown and then analyzed by SDS-PAGE, followed by autoradiography. Apparent molecular masses (in kDa) are indicated. Genes inferred to be present from complementation analysis (Fig. 1) are shown at the bottom of the figure. The FlaQII' protein (which differs slightly at the C terminus from the natural FlaQII protein; see text) and the FlaAII.3 protein are obscured by plasmid-encoded proteins but can be seen in the two-dimensional gels of Fig. 3. Note that FlaR and a protein of slightly lower apparent molecular mass (unmarked arrow) are either both present or both absent (see text).

analyzed by the minicell method (Fig. 7 and 8A). With pDB7, we could detect the $flaBIII_E$, $flaC_E$, $flaO_E$, and $flaE_E$ products, consistent with the complementation data. However, even though an $flaAII_E$ mutant could be complemented by pDB7, neither the $flaAI_E$ nor the $flaAII_E$ product was detected; this difference was noted previously by Bartlett and Matsumura (3) and may arise because $flaAI_E$ and $flaAII_E$ are under control of their own promoter rather than the *lacUV5* promoter.

With another plasmid, pCK210, which has been shown to contain $flaAI_E$ and $flaAII_E$ (17), we could readily detect the $flaAII_E$ product in minicell preparations (Fig. 7 and 8B). Although Kuo and Koshland (17) were able to detect the $flaAI_E$ product also with this plasmid, we were unable to do so.

Homologous products of *E. coli* and *S. typhimurium* showed generally similar electrophoretic mobilities (Fig. 7) and isoelectric points (Fig. 3 and 8). The most significant differences were that (i) $FlaE_E$ had an apparent molecular mass about 6 kDa greater than that of FlaR and (ii) $FlaC_E$ was appreciably more basic than FlaAIII.

DISCUSSION

Flagellar gene region III of *S. typhimurium* contains at least 16 genes; for only a few of these genes have functions been identified. As part of an effort to learn more about the genes in this region, we cloned six of them (*flaAII.3*, *flaAIII*, *flaS*, *flaR*, *flaQI*, and *flaQII*) and identified their products in minicell preparations (Fig. 2 and 3). A direct comparison between the homologous gene products of *S. typhimurium*

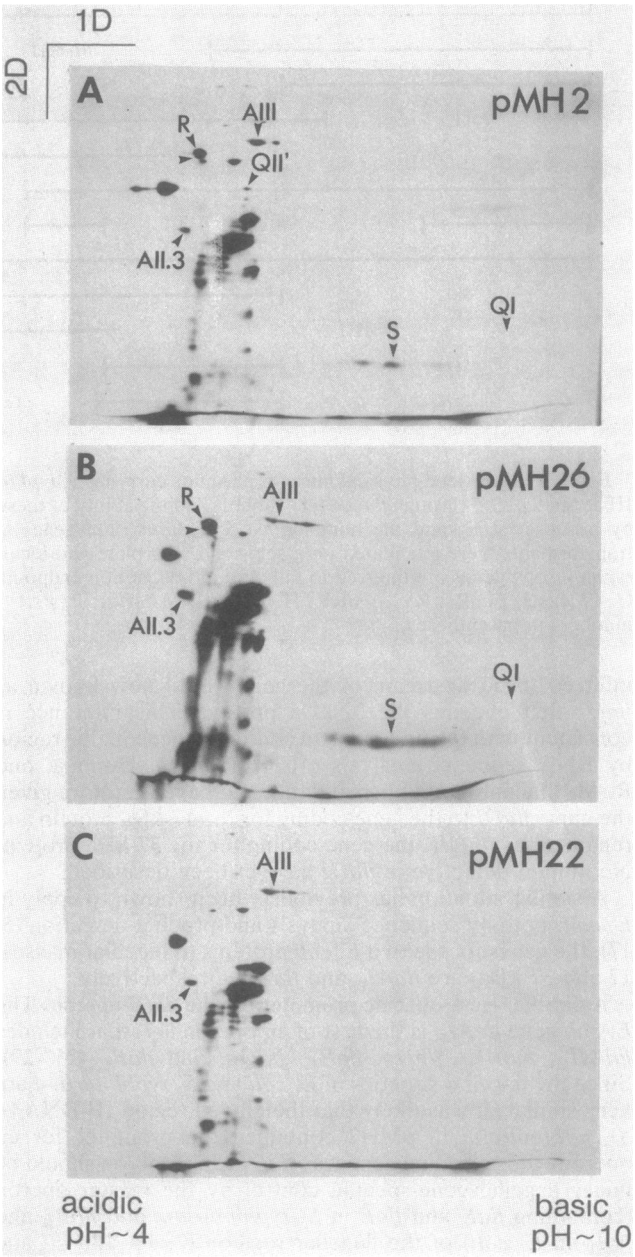


FIG. 3. Two-dimensional gel electrophoretograms of radiolabeled *S. typhimurium* flagellar proteins synthesized from plasmids in a minicell strain. (A) FlaAII.3, FlaAIII, FlaS, FlaR, FlaQI, and FlaQII' proteins detected from pMH2; (B) FlaAII.3, FlaAIII, FlaS, FlaR, and FlaQI detected from pMH26; (C) FlaAII.3 and FlaAIII detected from pMH22. The spot corresponding to FlaQI is visible at longer exposures at the position indicated. The acidic protein at the same apparent molecular mass as FlaQII' is a plasmid-encoded protein; it was absent in other plasmids that were shown by complementation to contain FlaQII'. The protein that appears along with FlaR (see text) is indicated by an unmarked arrow. The samples were analyzed by isoelectric focusing (one dimensional [1D]), followed by SDS-PAGE (two-dimensional [2D]), and detected by fluorography.

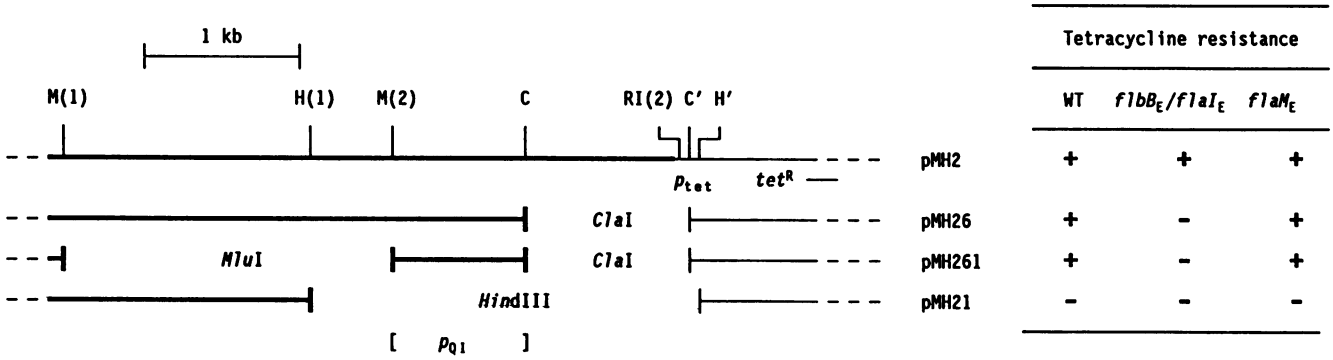


FIG. 4. Analysis of the location and properties of the *flaQI* promoter (p_{QI}). In the hybrid plasmids shown, DNA to the left of the *Eco*RI restriction site RI(2) derives from *S. typhimurium*, while DNA to the right derives from pBR322. In pMH2, the tetracycline resistance promoter (p_{tet}) is intact; in the other plasmids shown, it has been destroyed by the deletions shown. The ability of these plasmids to convey tetracycline resistance to various hosts, as a result of expression of the Tet^r gene, is shown on the right. From these results, the flagellar-gene-specific promoter (p_{QI}) is inferred to be within the restriction fragment M(2)-C. Symbols for restriction sites are defined in the legend to Fig. 1; primes (') indicate sites within pBR322 DNA. WT, Wild type.

and *E. coli* revealed that they are quite similar with respect to apparent molecular mass and isoelectric point.

We have previously carried out a biochemical analysis of the hook-basal body complex of *S. typhimurium* (1) and identified several of its components. A comparison of two-dimensional gels of the complex and of the products of the genes cloned in the present study indicates that none of the latter proteins are components of that complex.

Another general comment may be made regarding these products. With the possible exception of FlaR, we found no evidence for the processing that occurs with the proteins of the outer rings of the basal body (8, 10); it seems unlikely, therefore, that they are destined for export across the membrane, at least by the general signal-dependent pathway.

The analysis identified for the first time an *S. typhimurium* gene that is located immediately upstream of *flaQ* and had been missed in genetic studies. We have named this gene *flaQI* and have renamed the *flaQ* gene *flaQII*. The presence of *flaQI* had been expected from a study of the homologous region in *E. coli* (17) and also from the *S. typhimurium* DNA sequence (M. Kihara, M. Homma, and R. M. Macnab, unpublished data).

Neither *flaQI* nor the homologous *E. coli* gene, $flaAI_E$, have yet been detected by genetic analysis, i.e., no Fla^- mutants have been isolated and shown to have defects in those genes. However, $flaAI_E$ (and presumably *flaQI*) fall within the same operon as genes known to be involved in flagellar function, and it is well established that flagellar genes constitute a dedicated regulon of such operons (13, 14), within which there is no known example of a nonflagellar gene. It therefore seems extremely likely that $flaAI_E$ and *flaQI* do play a role in flagellar regulation, assembly, or function and that sooner or later a mutant phenotype associated with them will be established.

The location of the genes on a physical map can be inferred from the following results, with the assumption that genes within an operon are closely linked but do not overlap. (i) From the complementation ability of various deletion derivatives, it was determined which restriction sites lie within each gene (Fig. 1); also, it is known that the *Eco*RI site that terminates the insert actually lies just inside the 3' end of the *flaQII* gene. (ii) The length of each gene was estimated from the molecular mass of its product, as detected in minicells (Fig. 2). In this way, the genes could be assigned on the physical map (Fig. 9A), with the restriction site data and the gene sizes providing a self-consistent description. A similar map for the *E. coli* genes is presented in Fig. 9B, based on information from this and previous studies (3, 17). The physical locations of the genes on the maps of *S. typhimurium* and *E. coli* correspond well, al-

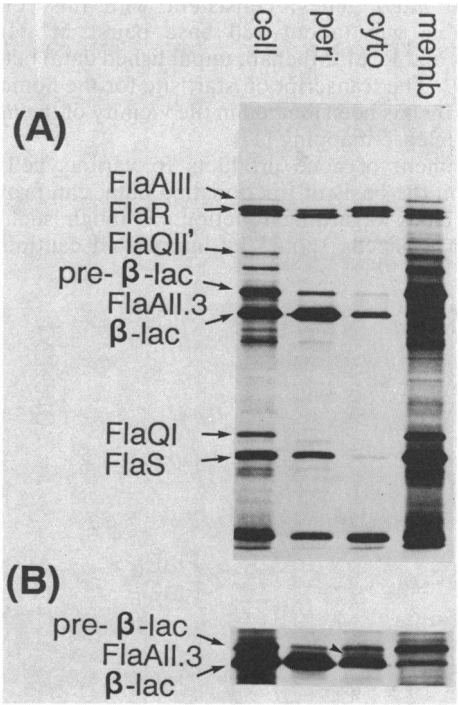


FIG. 5. Fractionation of the FlaAII.3, FlaAIII, FlaS, FlaR, FlaQI, and FlaQII' proteins of *S. typhimurium*. Radiolabeled minicells containing plasmid pMH1 were fractionated as described in the text and analyzed by SDS-PAGE. Cell, Whole-cell sample; peri, periplasmic fraction; cyto, cytoplasmic fraction, memb, membrane fraction. Pre-β-lac and β-lac are the precursor and mature forms of the plasmid-encoded β-lactamase protein, and (as expected) they appeared predominantly in the membrane and periplasmic fractions, respectively. The FlaAII.3 protein is not readily seen in autoradiogram (A), but in autoradiogram (B) of a different sample, it is present in the cytoplasmic fraction (arrow) and essentially absent from the membrane fraction.

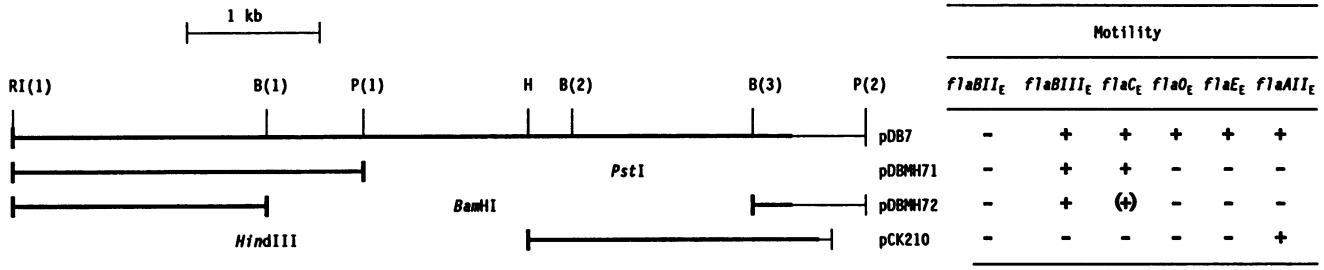


FIG. 6. Linearized physical maps of plasmids carrying *E. coli* DNA (thick horizontal line) in the vicinity of flagellar region III genes *flaBIII*$_E$ through *flaAII*$_E$ (cf. Table 2) and the ability of these plasmids to confer motility on *E. coli* mutants. Single colonies of kanamycin-resistant (for pDB7, pDBMH71, and pDBMH72) or ampicillin-resistant (for pCK210) transformants were inoculated on a semisolid agar plate and incubated at 37°C for 10 h. Restriction endonuclease sites are described in the legend to Fig. 1. B, *Bam*HI. DNA from phage λ is indicated by thin lines. The observed complementation of a *flaC*$_E$ mutant by pDBMH72 is not expected, at least in *trans*, since this plasmid does not synthesize a full-length *FlaC*$_E$ product (see Fig. 7); the reason for this result is unclear, but a similar result has been observed by Bartlett and Matsumura (3).

though restriction sites differ substantially, indicating some divergence between the DNA sequences.

On the basis of the organization of *E. coli* flagellar genes, the *flaAII.3*, *flaAIII*, *flaS*, and *flaR* genes and the *flaQI* and *flaQII* genes of *S. typhimurium* should belong to separate operons, i.e., the *flaAII.I* and *flaQI* operons, respectively (17, 25, 29). A flagellar-gene-specific promoter was located to a 0.7-kilobase fragment [M(2)-C in Fig. 1 and 4], and it must be the promoter for the *flaQI* operon, lying between the *flaR* and *flaQI* genes; consistent with this, there is an appreciable gap (about 130 base pairs; M. Kihara, M. Homma, and R. M. Macnab, unpublished data) between *flaR* and *flaQI*. The transcription start site for the homologous *E. coli* operon has been located in the vicinity of the *flaAI*$_E$ gene by S1 nuclease mapping (17).

Assignment of gene products to various cell compartments, on the basis of fractionation data, can provide some general clues regarding function, although such data obtained in minicells should be interpreted cautiously, since the natural destination of a protein may or may not be available and the observed fractionation may reflect the general biochemical character of the protein rather than a highly specific location. From other studies, the roles of two of the five cloned genes, *flaQII* and *flaR*, are understood to

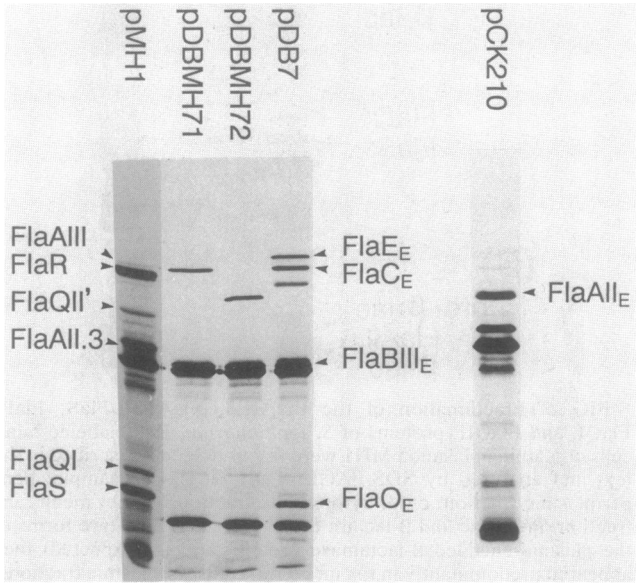


FIG. 7. Identification of products of homologous region III genes of *E. coli* and *S. typhimurium* (by the same methods described in the legend to Fig. 2). Plasmid pMH1 contains *S. typhimurium* DNA, and the others contain *E. coli* DNA.

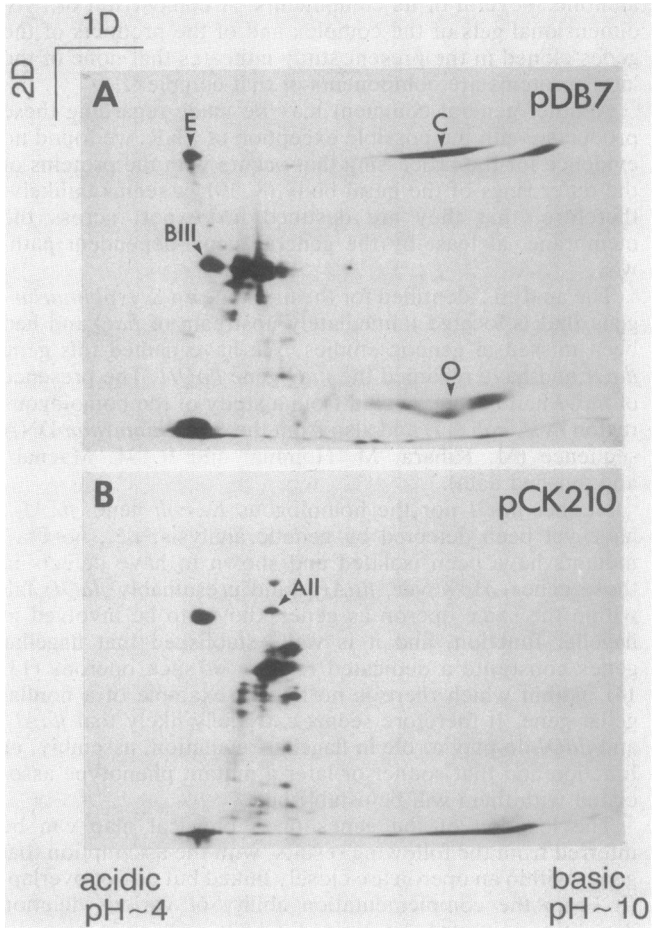


FIG. 8. Two-dimensional gel electrophoretograms of radiolabeled *E. coli* flagellar proteins synthesized from plasmids in a minicell strain. (A) FlaBIII$_E$, FlaC$_E$, FlaO$_E$, and FlaE$_E$ proteins detected from pDB7. (B) FlaAII$_E$ protein detected from pCK210; the FlaAI$_E$ protein which should be synthesized by this plasmid (17) could not be detected. For methods, see the legend to Fig. 3.

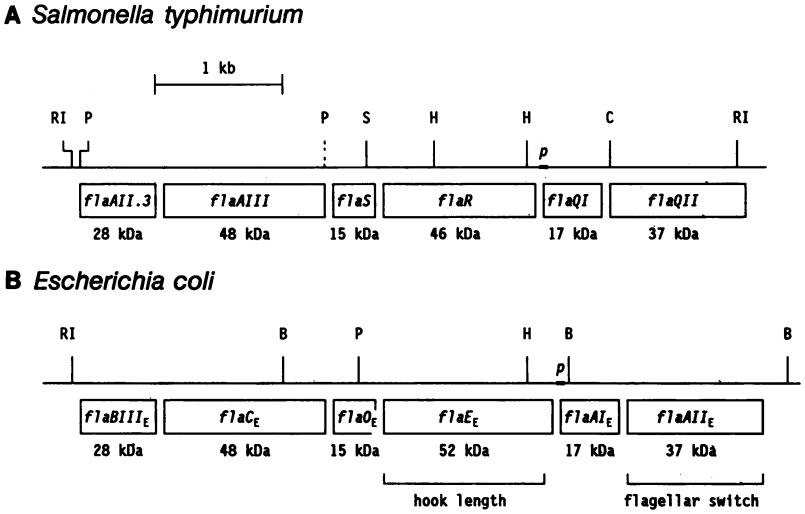


FIG. 9. Correspondence between region III genes and their products and location of the genes on the chromosome. (A) *S. typhimurium* genes *flaAII.3* through *flaQII*; (B) *E. coli* genes *flaBIII*$_E$ through *flaAII*$_E$. Homologous genes are shown in register. Symbols for restriction sites are described in the legends to Fig. 1 and 6. The intergenic region between *flaR* and *flaQI* (*flaE*$_E$ and *flaAI*$_E$) contains a flagellar-gene-specific promotor (*p*). In panel A, the 3′ end of the *flaQII* gene extends just beyond the *Eco*RI site that terminated the *S. typhimurium* DNA insert in plasmids pMH2, etc. The *Pst*I site (dashed line in panel A) is outside *flaS* but could either be in the *flaAIII* gene or in the intergenic region between *flaAIII* and *flaS*. The roles of FlaR/FlaE$_E$ and FlaQII/FlaAII$_E$ are indicated; the roles of the other proteins are unknown.

some degree; the roles of the remaining four are totally unknown. *flaQII* is one of three flagellar genes whose products are believed, on the basis of mutant phenotype and suppression analysis, to form a multisubunit switch complex that determines the counterclockwise versus clockwise sense of flagellar rotation (34, 35). (The other two switch genes are *flaAII.2* and *flaN*, both of which are also in region III [Table 2] and lie on either side of the fragment cloned in the present study.) The complex is probably associated with the M ring of the flagellar basal body, which is located in the cytoplasmic membrane (6). Genetic suppression evidence suggests that the switch complex is also exposed to the cytoplasm, with its switch state controlled by the binding of CheY or CheZ, known cytoplasmic components of the sensory system for chemotaxis (4, 18, 24, 27, 33). We found the FlaQII′ protein in the membrane fraction (Fig. 5), whereas Clegg and Koshland (using a generally similar fractionation procedure; 5) found the homologous *E. coli* protein, FlaAII$_E$, in the cytoplasmic fraction. It seems unlikely that the small C-terminal substitution of FlaQII′ is responsible for this difference. With the assumption that it is not, the difference would be hard to explain if the protein were integral to the cell membrane; however, a peripheral membrane protein with a less tenacious association with the membrane might fractionate differently as a result of small variations in the experimental conditions used. The idea that FlaQII (or FlaAII$_E$) is peripheral to the cell membrane is consistent with its role in the flagellar switch complex described above.

The helical flagellar filament that propels the cell is connected to the basal body via the hook, a structure that, unlike the filament, has a well-defined length. *flaR* mutants have hooks of indeterminate length (polyhooks), indicating that the role of FlaR is to ensure that the process of assembly of hook protein is terminated appropriately (26, 30). How it does this is not understood at all. We found that FlaR fractionated principally with the cytoplasm. If the cytoplasm is the natural location for FlaR, this may indicate a mechanism involving control of export rather than control of assembly at the hook structure itself.

Mutations in *flaAII.3*, *flaAIII*, and *flaS* cause a total failure of basal body assembly; no detectable flagellar structure is produced (31, 32). The fact that FlaAIII and FlaS were localized exclusively or primarily to the membrane fraction makes it unlikely that they control flagellar assembly at the level of control of gene expression. We speculate that FlaAIII and FlaS (probably in conjunction with other flagellar proteins) form a structure which surrounds and supports the basal body structure. FlaQI also was found in the membrane fraction, but until a mutant phenotype for this gene is determined, it is difficult to comment about its function. FlaAII.3 was predominantly in the cytoplasm, leaving open the possibility that it may have a regulatory role.

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