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SHORT COMMUNICATION

Genetic Analysis of Three Additional *fla* Genes in *Salmonella typhimurium*

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In *Salmonella typhimurium*, 27 *fla* genes responsible for formation of flagella have been identified and assigned to three regions on the genetic map, termed *fla* regions I to III. By genetic analysis of 1984 non-flagellate mutants obtained from a phase-1 stable strain of *S. typhimurium*, SJW1103, three additional *fla* genes were identified; one, termed *flaW*, was assigned to *fla* region I and the other two, termed *flaV* and *flaX*, to *fla* region III. By intergeneric complementation tests, the *flaW*, *flaV* and *flaX* genes were shown to be functionally homologous with *flaS*, *flbC* and *flaP* of *Escherichia coli*, respectively. Electron microscopy showed that *flaW* and *flaV* mutants carried hook–basal body structures.

INTRODUCTION

The bacterial flagellum is composed of a flagellar filament, a hook and a basal body (DePamphilis & Adler, 1971). The most extensive genetic analysis of the formation of flagella has been in *Salmonella typhimurium* and *Escherichia coli* (Iino, 1977; Silverman & Simon, 1977). Except for *H1* and *H2* in *Salmonella* and *hag* in *E. coli*, which are the structural genes for flagellin, the component protein of flagellar filaments, all the other genes responsible for the formation of flagella are termed *fla*. So far, nearly 30 *fla* genes have been found in both bacterial species (Kutsukake *et al.*, 1980; Komeda *et al.*, 1980). They have been assigned to three regions of the genome in both species: the region I *fla* genes near *pyrC*, the region II *fla* genes between *aroD* and *uvrC*, and the region III *fla* genes between *uvrC* and *supD* (Silverman & Simon, 1973; Komeda *et al.*, 1978; K. Kutsukake & I. Iino, unpublished results). Intergeneric complementation tests have revealed functional homology between most *fla* genes of the two species (Kutsukake *et al.*, 1980). However, genes functionally corresponding to *flaP*, *flaS* and *flbC* (*flb* is equivalent to *fla*) of *E. coli* had not been identified in *S. typhimurium* until the present study.

METHODS

Bacteria and media. *S. typhimurium* SJW1103 was used as the parent strain for the isolation of non-flagellate (*fla*) mutants. Strain SJW1103 is a derivative of *S. typhimurium* TM2, made phase-1 stable by transducing a deletion covering *rh2* and *H2* genes from *S. typhimurium* SJW916 *H2–203* (Yamaguchi *et al.*, 1984). The single site and deletion mutants used as standards of already established *fla* genes are also derivatives of SJW1103. Defective

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regions of some deletion mutants important for the present mapping are shown in Fig. 1. *S. typhimurium* strains SJ10002 and SJ10004 (Kutsukake *et al.*, 1980), used for the construction of restriction-negative P1-sensitive *fla* mutants, were *pyrC138* and *hisC527* derivatives, respectively, of *Salmonella* strain SL213 (Enomoto & Stocker, 1974). Non-flagellate derivatives of *E. coli* K12, including YK1101 *flbC*, YK4144 *flaP* and YK4429 *flaS*, were supplied by Dr Y. Komeda (Tokyo University, Tokyo, Japan).

The compositions of nutrient broth, nutrient agar and semisolid medium were described by Yamaguchi *et al.* (1972).

Isolation of non-flagellate mutants. The flagellotropic phage χ was used, as described previously (Yamaguchi *et al.*, 1972).

Test for complementation and recombination. Complementation and recombination between pairs of *Salmonella fla* mutants were examined by P22-mediated transduction. The transduction mixture was streaked in lines on semisolid medium, and the production of trails (abortive transductants) or of swarms (complete transductants) after overnight incubation was used as the criterion for complementation or recombination, respectively (Yamaguchi *et al.*, 1972).

For the intergeneric complementation tests, restriction-negative P1-sensitive *fla* strains of *Salmonella* were constructed by transducing *fla* alleles into P1-sensitive *S. typhimurium* strains SJ10002 or SJ10004. The *fla* alleles in region I were cotransduced with $pyrC^+$ into SJ10002 by phage P22, and the *fla* alleles of region III were transduced into SJ10004 with P22, making use of the Tn*10* inserted near the *fla* genes by the method of Kutsukake *et al.* (1980). The intergeneric complementation tests between *fla* mutants of *S. typhimurium* and *E. coli* were performed with P1*kc* according to Enomoto & Stocker (1974).

Electron microscopy. The presence or absence of flagellar basal structures in *fla* mutants was determined by the method of Suzuki *et al.* (1978). Prepared samples were negatively stained with 1% (w/v) potassium phosphotungstate (pH 7·2) and observed in a JEM100C electron microscope.

RESULTS AND DISCUSSION

Identification of new fla complementation groups

Using the flagellotropic phage χ as the selecting agent, 1984 spontaneous non-flagellate mutants were isolated from *S. typhimurium* SJW1103. They were subjected to the recombination test with long-deletion mutants to determine in which *fla* region their mutational sites were located; 487 clones were assigned to *fla* region I, 325 clones to region II and 1172 to region III. Following complementation tests with *fla* mutants for known *fla* complementation groups, most of the clones were assigned to a known group. However, 20 clones in region I and 19 clones in region III did not belong to any known *fla* group. Reciprocal complementation tests showed that those of region I constituted a single complementation group, which we termed gene *flaW*, while those of region III constituted two groups, one of 5 clones, which we termed gene *flaV*, and one of 14 clones, which we termed gene *flaX*.

Deletion mapping of the new fla genes

To determine the positions of the new *fla* genes within the *fla* regions, deletion mapping was carried out by transduction. Transductions were carried out from mutants of the new *fla* genes to mutants containing known deletions. *flaW* mapped between *flaFX* and *flaU* in region I, *flaV* between *flaAI* and *H1* in region III, and *flaX* at the left end of region III (Fig. 1).

Functional homology of the new fla genes with E. coli fla genes

There is a great deal of similarity in the distribution of *fla* genes on the chromosome of *Salmonella* and of *E. coli* (Iino, 1977; Silverman & Simon, 1977). Comparing the arrangement of *fla* genes of *S. typhimurium* with that of *E. coli* K12 (Komeda *et al.*, 1980), the new genes *flaW*, *flaV* and *flaX* of *S. typhimurium* are likely to correspond to the *E. coli* genes *flaS*, *flbC* and *flaP*, respectively (Fig. 2). To examine functional homology between these genes, intergeneric complementation tests were carried out by P1-mediated transduction. P1-sensitive *Salmonella* strains carrying *flaW*, *flaV* or *flaX* mutations were constructed by transducing the mutations into P1-sensitive SJ10002 or SJ10004; *flaW2391* was introduced into SJ10002 from strain SJW2160, and *flaV2380* of SJW2149 and *flaX1181* of SJW2021 were introduced into SJ10004. Using these P1-sensitive *fla* strains as recipients, intergeneric transductions were carried out from *E. coli fla* mutants representing all the known *fla* groups. The only *E. coli* mutants that did not complement

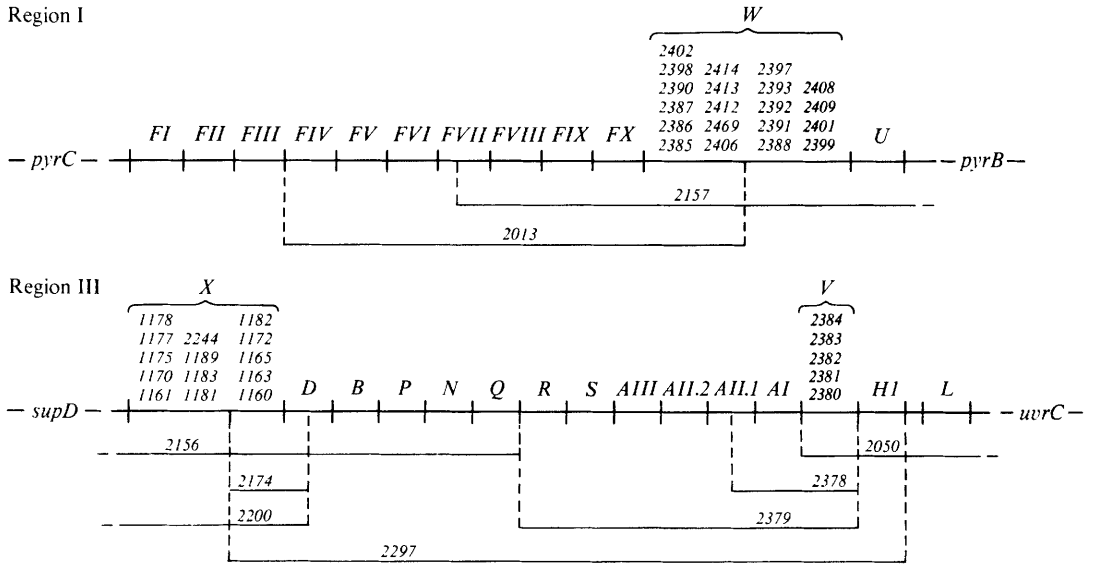

Fig. 1. Mutational sites of *flaW*, *flaV* and *flaX* mutants in *fla* regions I and III of *Salmonella typhimurium*. For the other *fla* genes, only cistron designations are given. *H1* is the structural gene for flagellin. Horizontal lines represent the extent of deletions that are important for the mapping of new *fla* genes.

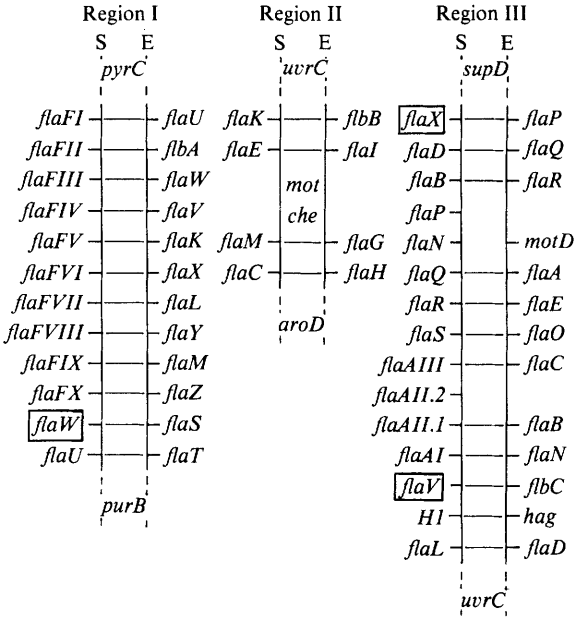

Fig. 2. Functional homology between *fla* genes of *Salmonella typhimurium* (S) and *Escherichia coli* (E). Pairs of homologous genes are connected by a horizontal line. (Figure constructed from Kutsukake *et al.*, 1980, Komeda *et al.*, 1980, and this study.)

the *flaW*, *flaV* and *flaX* mutants of *S. typhimurium* were *flaS*, *flbC* and *flaP*, respectively, which indicates functional homology between these genes in the two bacteria.

Presence or absence of incomplete flagellar structures in mutants of the new fla genes

Mutants of some *fla* genes in both *S. typhimurium* (Suzuki *et al.*, 1978) and *E. coli* (Suzuki *et al.*, 1981) carry incomplete flagellar structures specific for mutated genes. In *E. coli*, mutants of the

flaS and *flbC* genes carry hook–basal body complexes apparently indistinguishable from those of intact flagella, while mutants of the *flaP* gene lack a detectable flagellar structure (Suzuki *et al.*, 1981). Thus, presence or absence of incomplete flagellar structures in *flaW*, *flaV* and *flaX* mutants of *S. typhimurium* was determined. The mutants examined were SJW2160 (*flaW2391*), SJW2177 (*flaW2408*), SJW2149 (*flaV2380*), SJW2150 (*flaV2381*), SJW160 (*flaX1160*) and SJW161 (*flaX1161*). As expected, in *flaW* and *flaV* mutants apparently complete hook–basal body complexes were observed, while no flagellar structure was detected in *flaX* mutants. These results support the conclusion drawn from the complementation tests that the *flaW*, *flaV* and *flaX* genes correspond to the *flaS*, *flbC* and *flaP* genes of *E. coli*, respectively.

Recently, we have shown that the intact hook carries three kinds of protein at its tip (hook-associated protein or HAP), termed HAP1, HAP2 and HAP3, and that hooks from *flaV* mutants lack HAP2 and those from *flaW* mutants lack HAP1 and HAP2 (Homma *et al.*, 1984*a*). We have also observed that *flaW* and *flaV* mutants excrete intact flagellin into the medium (Homma *et al.*, 1984*b*). These observations strongly suggest that the *flaW* and *flaV* genes are concerned with the production of HAPs and play an essential role in the initiation of assembly of flagellin molecules into a filament at the tip of the hook.

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REFERENCES

DEPAMPHILIS, M. L. & ADLER, J. (1971). Attachment of flagellar basal bodies to the cell envelope: specific attachment to the outer, lipopolysaccharide membrane and the cytoplasmic membrane. *Journal of Bacteriology* **105**, 396–407.

ENOMOTO, M. & STOCKER, B. A. D. (1974). Transduction by phage P1*kc* in *Salmonella typhimurium*. *Virology* **60**, 503–514.

HOMMA, M., KUTSUKAKE, K., IINO, T. & YAMAGUCHI, S. (1984*a*). Hook-associated proteins essential for flagellar filament formation in *Salmonella typhimurium*. *Journal of Bacteriology* **157**, 100–108.

HOMMA, M., FUJITA, H., YAMAGUCHI, S. & IINO, T. (1984*b*). Excretion of unassembled flagellin by *Salmonella typhimurium* mutants deficient in hook-associated proteins. *Journal of Bacteriology* (in the Press).

IINO, T. (1977). Genetics of structure and function of bacterial flagella. *Annual Review of Genetics* **11**, 161–182.

KOMEDA, Y., SILVERMAN, M., MATSUMURA, P. & SIMON, M. (1978). Genes for the hook–basal body proteins of flagellar apparatus in *Escherichia coli*. *Journal of Bacteriology* **134**, 655–667.

KOMEDA, Y., KUTSUKAKE, K. & IINO, T. (1980). Definition of additional flagellar genes in *Escherichia coli* K12. *Genetics* **94**, 277–290.

KUTSUKAKE, K., IINO, T., KOMEDA, Y. & YAMAGUCHI, S. (1980). Functional homology of *fla* genes between *Salmonella typhimurium* and *Escherichia coli*. *Molecular and General Genetics* **178**, 59–67.

SILVERMAN, M. & SIMON, M. (1973). Genetic analysis of flagellar mutants in *Escherichia coli*. *Journal of Bacteriology* **113**, 105–113.

SILVERMAN, M. & SIMON, M. (1977). Bacterial flagella. *Annual Review of Microbiology* **31**, 397–419.

SUZUKI, T. & KOMEDA, Y. (1981). Incomplete flagellar structures in *Escherichia coli* mutants. *Journal of Bacteriology* **145**, 1036–1041.

SUZUKI, T., IINO, T., HORIGUCHI, T. & YAMAGUCHI, S. (1978). Incomplete flagellar structures in non-flagellate mutants of *Salmonella typhimurium*. *Journal of Bacteriology* **133**, 904–915.

YAMAGUCHI, S., IINO, T., HORIGUCHI, T. & OHTA, K. (1972). Genetic analysis of *fla* and *mot* cistrons closely linked to *H1* in *Salmonella abortusequi* and its derivatives. *Journal of General Microbiology* **70**, 59–75.

YAMAGUCHI, S., FUJITA, H., SUGATA, K., TAIRA, T. & IINO, T. (1984). Genetic analysis of *H2*, the structural gene for phase-2 flagellin in *Salmonella*. *Journal of General Microbiology* **130**, 255–265.