Genetic Analysis of *H1*, the Structural Gene for Phase-1 Flagellin in *Salmonella*

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SUMMARY

For the mapping of *H1*, the structural gene for phase-1 flagellin in *Salmonella*, spontaneous non-flagellate *H1* mutants were isolated from a phase-1 stable derivative, SJ925 *H1*-*g*₁*g*₂*g*₃*t*, of *Salmonella abortusequi*. First, mapping was carried out with the deletion mutants among them by P22 phage-mediated transduction. Mutants of *flaAI* and *flaL*, adjoining opposite sides of *H1*, were also included in the mapping. As the result, *H1* was divided into 16 segments by 15 deletions. Mapping by recombination frequencies was then carried out using representative *H1* mutants. Comparison of the two maps showed that 14 consecutive segments near *flaL* covered about 70 % of the non-flagellate *H1* mutational sites, although they were confined to a quarter of *H1* in the recombination map. The other two segments were found to occupy the remaining three quarters of *H1*. By use of the deletion map, the sites of three phase-1 curly and three *ah1*⁻ mutations were determined. The curly mutational sites were mapped in the segment second from the *flaAI* side and the *ah1*⁻ mutational sites in the segments near the *flaL* side. To ascertain approximate positions of the areas determining the phase-1 antigen specificities, their arrangement relative to a curly mutational site, *curly-2*, and *H1*-linked *fla* genes was examined by three-point crosses. From the results, all the antigenic specificity-determining areas examined were located between *flaAI* and *curly-2* in the following order: *flaAI*-*g*₂*g*₁*g*₄-(*g*₃*g*₅*f**m**t*)-*curly-2*-*flaL*.

INTRODUCTION

In *Salmonella*, *H1* and *H2* were originally defined as the genes determining antigenic specificities of phase-1 and phase-2 flagella, respectively (Lederberg & Edwards, 1953). As it was later shown that the unit of flagellar antigen is the protein termed 'flagellin', which is the sole constituent of flagellar filaments, *H1* and *H2* are now defined as the structural genes for flagellin of the respective phases (Iino, 1969). It is known that not only antigenic specificities but also the shape of the flagellar filaments are primarily determined by the *H* genes (Iino, 1962; Iino & Mitani, 1966, 1967; Enomoto & Iino, 1966).

Several papers have so far described the fine-structure analysis of the *H* genes. By three-point crosses, Joys & Stocker (1969) attempted to determine the order of the sites of a curly mutation causing shortening of the wavelength of flagella and of mutations causing alterations in flagellar antigenic specificity, but could not reach a conclusive result. Yamaguchi & Iino (1969) demonstrated intragenic recombination between *H1* alleles

specifying various forms of the g-complex antigens and mapped some antigenically-important areas of the *Hr* gene in a linear array. Yamaguchi *et al.* (1972) reported a deletion map of *Hr* in which the gene was divided into four segments. To approach the problem of how the *H* genes determine flagellar characters, more detailed genetic maps of these genes are indispensable.

In this study, a map of *Hr* was constructed with non-flagellate *Hr* mutants isolated from a phase-I stable derivative of *Salmonella abortusequi*. Further, by use of the map the locations of mutations responsible for phase-I curly flagella and of mutations which have been assumed to be in *ahr*, the region controlling the activity of *Hr*, were determined. An attempt was also made to allocate antigenic specificity-determining areas within *Hr*.

METHODS

Bacteria and phages. The characteristics and derivation of the *Salmonella* strains used are described in Table 1.

Phage χ M8 (Sasaki, 1962; Yamaguchi, 1968) is a host-range mutant of phage χ . Phage χ attacks motile *Salmonella* organisms except for some specific serotypes including g-complex antigenic *Salmonella* (Meynell, 1961). The host-range of phage χ M8 includes motile organisms with g-complex antigens.

Phage P22 was used as the mediator in transduction experiments.

Phage lysates were prepared by the soft-agar layer method and titrated on *Salmonella* strain SJ925.

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

Isolation of non-flagellate mutants. Spontaneous non-flagellate mutants were isolated from a motile strain, SJ925, by the combined use of phage χ M8 and semi-solid medium. The procedure was described by Yamaguchi *et al.* (1972).

Test for complementation and recombination. Complementation and recombination between pairs of non-flagellate mutants were examined by transduction. Production of trails (abortive transductants) and swarms (complete transductants) following transduction were used as the criteria for complementation and recombination, respectively (Yamaguchi *et al.* 1972).

For two-factor crosses, equal volumes of P22 phage suspension adjusted to 10^{10} infective units/ml and bacterial suspension containing 10^9 organisms/ml were mixed. After 10 min adsorption, 0.1 ml of the mixture was diluted 10- or 50-fold, and the whole quantity of the dilution was streaked on semi-solid medium plates. After overnight incubation at 37 °C, the number of recombinants appearing as swarms was counted.

Recombination frequencies between the donor and the recipient mutational sites were expressed as percentages of the number of recombinants obtained when phage grown on the wild-type strain was used with the same recipients.

Identification of the antigenic specificity of flagella. Antigenic specificities of flagella were identified by slide agglutination with anti-factor(s) sera prepared according to Yamaguchi *et al.* (1969).

Description of bacterial strains. The mutant number, assigned from 2101 onwards for the non-flagellate *Hr* mutants derived from *Salmonella* strain SJ925, was also used to designate the corresponding mutational site.

For the description of the flagellar antigen type, the factor composition previously reported (Yamaguchi & Iino, 1969) was used, e.g. 'g₁, g₂, g₃, t' for that of strain SJ925, which

Table 1. *Bacteria*

Strain no.	Mutant no.	Characteristics and derivation
SJ925		Motile phase-I stable derivative of <i>Salmonella abortusequi</i> SJ241 with <i>Hr-g₁,g₂,g₃,t</i> transduced from <i>Salmonella</i> sp. SJ6 (Yamaguchi <i>et al.</i> 1972)
SJ1585	<i>flaL204</i>	Mutants of SJ929, a derivative of SJ241 with <i>Hr-g₃,g₄,f</i> transduced from <i>S. abony</i> NCTC1729 (Yamaguchi <i>et al.</i> 1972)
SJ1727	<i>fla373</i>	
SJW310	<i>flaAI1310</i>	Mutants of <i>S. abortusequi</i> SJ241 <i>Hr-a</i> (Yamaguchi <i>et al.</i> 1972)
SJW417	<i>Hr-1104</i>	
SJ1690	<i>flaL309</i>	Mutants of SJ930, a derivative of SJ241, with <i>Hr-g₁,g₂,g₄,g₅,m</i> transduced from <i>S. enteritidis</i> NCTC4196
SJ1632	<i>flaAI1251</i>	
SJ1891	<i>Hr-curly-1</i>	Phase-I curly mutants derived from SJ929 <i>Hr-g₃,g₄,f</i>
SJ1892	<i>Hr-curly-2</i>	
SJ1893	<i>Hr-curly-3</i>	
SW1061	<i>ah1-1</i>	O-H variants derived from <i>S. typhimurium</i> (Iino, 1961)
SW629	<i>ah1-2</i>	
SW547	<i>ah1-3</i>	

means that the flagellar antigen consists of factors g_1 , g_2 , g_3 and t . The chromosomal area determining the specificity of an antigenic factor was described by the italicized form of the symbol for the corresponding factor, e.g. g_1, g_2, g_3 , etc. The italicized symbol was also used to designate the *Hr* allele, e.g. *Hr-g₁,g₂,g₃,t* for that of strain SJ925, which means that the *Hr* allele carries antigenic specificity-determining areas g_1 , g_2 , g_3 and t .

RESULTS

Isolation and characterization of Hr, flaAI and flaL mutants

Using phage χ M8 as the selecting agent, 4247 spontaneous non-flagellate mutant clones were isolated from a phase-I stable strain, SJ925 *Hr-g₁,g₂,g₃,t*.

The non-flagellate phenotype is caused not only by mutation in *Hr* but also in any of the *fla* genes. As it has been found that among the 15 known *fla* genes, *flaAI* and *flaL* adjoin opposite sides of *Hr* (Yamaguchi *et al.* 1972), mutants of *flaAI* and *flaL* as well as *Hr* were looked for among those isolated.

To identify *Hr*, *flaAI* and *flaL* mutants, all the non-flagellate clones obtained were subjected to complementation tests with three strains, SJW417 *Hr-1104*, SJW310 *flaAI1310* and SJ1585 *flaL204*, respectively. These indicator strains were used as donors in transduction experiments. As a result, 67 clones were identified as *Hr* mutants, 14 as *flaAI*, 102 as *flaL*, and five as *Hr flaL*. Among them, 21 *Hr*, eight *flaAI*, 16 *flaL* and all five *Hr flaL* mutants were stable, and the others revertible (Table 2).

Ten mutants were chosen at random from each of the three complementation groups and examined in all pairwise combinations for complementation. In all the inter-cistronic crosses, complementation was perfect, i.e. as many trails as those in the crosses with the wild-type donor were produced. In intra-cistronic crosses no complementation occurred except for some crosses between *flaL* mutants, in which fewer than 50 % of the trails in the control crosses were produced.

Table 2. Characteristics of *Hr*, *flaAI*, and *flaL* mutants isolated from *Salmonella* strain SJ925 and used for deletion mapping

Complementation group	Mutant no.									
	Stable					Revertible				
<i>Hr</i>	2105	2110*	2111	2116		2101	2102	2103	2104	2106
	2121*	2122	2124	2128		2108	2109	2112	2113	2114
	2130*	2134*	2148*	2150*		2115	2117	2118	2119	2120
	2153*	2158*	2161	2163		2123	2126	2127	2129	2131
	2164	2169*	2170	2173		2132	2133	2135	2139	2142
	2177					2145	2147	2149	2151	2152
						2154	2160	2162	2165	2166
						2167	2168	2171	2172	2174
						2175	2176	2178	2179	2180
						2181				
<i>flaAI</i>	1477*	1479	1480	1481		1478	1483	1484	1485	1490
	1482*	1486*	1487	1488		1491				
<i>flaL</i>	1508	1510	1513	1515		1514	1521	1523		
	1516	1517*	1518	1519						
	1520	1522	1524	1531						
	1532*	1535*	1598	1599						
<i>Hr flaL</i>	2107*	2137*	2140*	2141*						
	2143*									

* Confirmed to be deletion mutants.

Deletion mapping of Hr and its vicinity

Deletion mapping was achieved by the following two series of crosses. First, using the mutants listed in Table 2, transductions were carried out from each revertible mutant to all the stable ones. Stable mutants failing to produce swarms by transduction from more than two revertible mutants were regarded as deletion mutants. As a result, ten *Hr*, three *flaAI*, three *flaL* and all five *Hr flaL* mutants were confirmed to be deletion mutants. Transductions were then carried out in all possible combinations among the deletion mutants. In these crosses, SJW310 *flaAI*1310, SJ1585 *flaL*204 and SJ1690 *flaL*309, whose mutations have been established to be deletions (Yamaguchi *et al.* 1972; Yamaguchi, unpublished), were also included. On the basis of their overlapping pattern, it was shown that all the *Hr*, *flaL* and *Hr flaL* deletions were arranged in a contiguous sequence, and the four *flaAI* deletions in another contiguous sequence.

Combining the results of these two series of crosses, a deletion map (Fig. 1) was constructed. *Hr* was divided into 16 segments, *flaAI* into six segments and *flaL* into eight segments. In this map, stable mutations which were not confirmed to be deletions were included in the group of single site mutations. The orientation of *flaAI* segments with respect to *Hr* was determined to be as shown in Fig. 1 by a three-point cross test between SJW478 *flaAI*1478 *Hr*-*g*₁, *g*₂, *g*₃, *t* and SJW310 *flaAI*1310 *Hr*-*a*, using the *Hr* character as unselected marker according to the method of Yamaguchi *et al.* (1972). Segments in the *Hr* gene were given consecutive numbers from 1 to 16 running from the right-hand (*flaL*) side to the left.

Mapping by recombination frequencies

In the deletion map (Fig. 1) the segments were arbitrarily shown as of equal length. To know more precisely the distribution of the mutational sites, mapping by recombination frequencies in two-factor crosses was carried out. The mutational site of strain SJW510

* Positions of the *flaAI* mutations which were not covered by any deletion were determined by three-point crosses with *snw310 flaAIr310 Hr-a* using *Hr* as unselected marker.

† Mutations in *Salmonella* strain *sn241 Hr-a*.

‡ Mutation in *Salmonella* strain *sn29 Hr-g₃g₄f*.

Fig. 1. Deletion map of *Hr*, *flaAI*, and *flaL* in strain *sn25*, a derivative of *Salmonella abortussequi*. Horizontal lines represent the extent of deletions. Mutations that were stable but yielded wild-type recombinants with all revertible mutations were included in the group of single site mutations. The length of each cistron and segment is arbitrary. Segments in *Hr* were numbered from right to left and the numbers represented by circled figures.

Table 3. *Recombination frequencies between strain SJW510 flaL1510 and non-flagellate H1 mutants*

For details, see Methods.

Recipient (mutant no.)	No. of motile recombinants in transduction from		Recombination frequency (B/A, %)
	SJ925 (wild-type) (A)	SJW510 <i>flaL1510</i> (B)	
<i>flaL1510</i>	1400	0	0
<i>H1-2129</i>	1050	157	15.0
<i>H1-2122</i>	990	161	16.3
<i>H1-2111</i>	1240	206	16.6
<i>H1-2149</i>	1040	184	17.7
<i>H1-2120</i>	1100	221	20.1
<i>H1-2116</i>	1220	250	20.5
<i>H1-2161</i>	950	278	29.3
<i>H1-2178</i>	1220	425	34.8
<i>flaAl1490</i>	1100	435	39.3

flaL1510, which is located in the segment of *flaL* nearest to *H1*, was used as the standard site. Transductions were carried out from strain SJW510 *flaL1510* to eight *H1* mutants chosen from various segments and strain SJW490 *flaAl1490* whose mutational site is located in the segment of *flaAl* nearest to *H1*. The number of motile recombinants appearing as swarms on semi-solid medium was counted. As a control, transduction was carried out from the wild-type strain SJ925 to each of the recipients. The relative recombination frequencies between *flaL1510* and the recipient mutational sites were calculated by taking the number of motile recombinants evoked in the respective control transductions as 100 %. From the data (Table 3) the map shown in the lower part of Fig. 2 was constructed. The mutational sites in *H1* were distributed in the range 15.0 to 34.8 %. Their relative order fully accorded with that in the deletion map. The main thing disclosed by comparison of the recombination map with the deletion map is that mutational sites located at segments 1 to 14 in the latter were concentrated in a region comprising a quarter of the *H1* gene, to its *flaL* side, in the former map. As shown in Fig. 1, about 70 % of the non-flagellate *H1* mutational sites were mapped in segments 1 to 14. Thus the quarter at the *flaL* side of the *H1* gene is considered to be 'hot' as regards the occurrence of spontaneous non-flagellate mutations compared with the other three quarters. It was shown that *H1-2116* and *H1-2161*, both of which were allocated to segment 15, mapped at positions 20.5 and 29.3 in the recombination map, respectively, indicating that segment 15 occupies most of the remaining three-quarters of the gene.

Mapping of mutational sites responsible for curly flagella

The genetic change of flagellar shape is due to mutation at sites in *H1* or *H2*; depending on which gene mutates, the mutant shape appears in either phase-1 or phase-2 (Iino, 1969). The most commonly occurring flagellar-shape mutants are curly; their flagella are characterized by a wavelength about half the normal one.

Using *H1* deletions, we attempted to locate the mutational sites of three phase-1 curly mutants, SJ1891 *H1-curly-1*, SJ1892 *H1-curly-2* and SJ1893 *H1-curly-3*, in the deletion map. Transductions were carried out from the curly mutants to all the *H1* deletion mutants. In the crosses with SJW682 *H1-2137*, SJW685 *H1-2140*, SJW666 *H1-2121* and SJW587 *H1-2107*, none of the curly mutants evoked swarms, while in other crosses they evoked swarms.

Fig. 2. Comparison between deletion and recombination maps of the *flaAI-H1-flaL* region. Above: Deletion map. Circled figures represent the segment numbers. Below: Recombination map. Figures below the horizontal line represent the distances from *flaL*1510, made proportional to recombination frequencies.

Fig. 3. Location of phase-I curly and *ahI*⁻ mutational sites in the *H1* gene.

Consequently, the three curly mutational sites were allocated to segment 15 where the four deletions overlap (Fig. 3).

Mapping of *ahI*⁻ mutations

O-H variation in some diphasic *Salmonella* strains has been attributed to a mutant allele of *ahI* (*H1*-activity controller) which is closely linked to but separable from antigen type determinants of the *H1* gene (Iino, 1961, 1969). The position of *ahI* relative to *H1* has not yet been determined.

By the same method as used in the mapping of curly mutational sites, the mutational

sites of three previously-reported *ahI*⁻ mutants of *Salmonella typhimurium*, SW1061 *ahI*-1, SW629 *ahI*-2 and SW547 *ahI*-3, were mapped. As shown in Fig. 3, they were allocated to three different segments near *flaL* within the *H1* region.

Mapping of antigenic specificity-determining areas

The flagellar antigen is a complex composed of several antigenic factors differing in specificity; that is, one flagellin molecule carries several distinct antigenic determinants. By intragenic recombination between *H1* alleles specifying various kinds of g-complex antigens, the following arrangement of some antigenic specificity-determining areas in the *H1* gene was disclosed (Yamaguchi & Iino, 1969):

$$\text{---flaAI---}\overbrace{g_2g_1g_4(g_3g_5f,m,t)}^{H1}\text{---flaL---}.$$

To combine this map with the present one, we attempted to determine the position of antigenic specificity-determining areas relative to a curly mutational site by the following three-point cross tests. Transductions were carried out from a curly mutant to *H1*-linked *fla* mutants derived from motile strains carrying different antigenic factor compositions, and hence different *H1* alleles, from those of the donor; then motile recombinants were selected on semi-solid medium and their antigenic factor compositions were examined.

As the donor curly mutant, SJ1892 *H1*-curly-2, whose *H1* allele is *H1*-*g*₃*g*₄*f*, curly, was used. Transduction was first carried out from the donor to a deletion mutant, SJ1726 *fla373*, whose deletion covers *flaAI*, *AII*, *AIII*, *B*, *D*, *N*, *P*, *Q* and *R* and whose *H1* allele is *H1*-*g*₁*g*₂*g*₃*t*. Among 57 motile transductants obtained, two clones carried the donor-type factor composition and 53 the recipient-type one, while the remaining two carried hybrid-type ones, that is, '*g*₁*g*₃*t*' (hybrid-type I) and '*g*₃*g*₄*t*' (hybrid-type II), respectively (Table 4). Assuming that all the recombinants resulted from two crossovers, one in the region between *fla373* and *curly*-2 and the other beyond *fla373*, the occurrence of the above four types of recombinants can be explained only when antigenic specificity-determining areas are located as shown in Table 5A. That is, the donor-type clones are considered to have resulted from two crossovers in regions 1 and 5 in Table 5A, the recipient-type ones in regions 1 and 2, the hybrid-type-I clone in regions 1 and 3, and hybrid-type-II clone in regions 1 and 4. Therefore, the following order of antigenic specificity-determining areas relative to *curly*-2 and *fla373* is inferred:

$$\text{---fla373---}g_2(g_1g_4)\text{---}(f,t)\text{---curly-2---}.$$

As *g*₃ is common to both donor and recipient *H1* alleles, its position cannot be inferred from this cross.

Transduction was next carried out from SJ1892, the same donor as above, to SJ1632 *flaAII251*, whose *H1* allele is *H1*-*g*₁*g*₂*g*₄*g*₅*m*. Among 104 motile transductants obtained, four carried the donor-type factor composition and 100 carried the recipient-type one. The occurrence of these two types of transductants is explained as shown in Table 5B. That is, crossovers in regions 1 and 3 resulted in the production of the donor-type recombinants and those in regions 1 and 2 resulted in the production of the recipient-type ones. Thus, the order

$$\text{---flaAII251---}(g_1g_2g_3g_5f,m)\text{---curly-2---}$$

is inferred. As *g*₄ is common to both donor and recipient *H1* alleles, its position cannot be inferred from this cross.

Table 4. *Flagellar antigens of recombinants obtained in crosses between a curly mutant and fla mutants carrying different Hr alleles*Transduction was carried out from the curly mutant to *fla* mutants.

Transduction		No. of recombinants			
Donor	Recipient	Donor-type*	Recipient-type*	Hybrid-type†	Total
SJ1892 <i>Hr</i> - <i>g</i> ₃ <i>g</i> ₄ <i>f</i> , <i>curly</i> -2	SJ1727 <i>fla</i> 373‡ <i>Hr</i> - <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₃ <i>t</i>	2	53	2	57
	SJ1632 <i>fla</i> III251 <i>Hr</i> - <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₄ <i>g</i> ₅ <i>m</i>	4	100	0	104

* Factor composition of the donor-type is '*g*₃*g*₄*f*' in both crosses, and those of the recipient-types are '*g*₁*g*₂*g*₄*t*' in the upper cross and '*g*₁*g*₂*g*₄*g*₅*m*' in the lower one.

† Factor compositions of the two clones obtained in the upper cross are '*g*₁*g*₃*t*' and '*g*₃*g*₄*t*', respectively.

‡ *fla*373 is a deletion covering *fla*AI, *fla*II, *fla*III, *B*, *D*, *N*, *P*, *Q*, and *R*.

Table 5. *Possible arrangement of antigenic specificity-determining areas in Hr with respect to curly and fla mutational sites, that explains the occurrence of the recombinants shown in Table 4*

Possible arrangement	Factor composition of recombinants	Crossover regions
(A) Transduction from SJ1892 <i>Hr</i> - <i>g</i> ₃ <i>g</i> ₄ <i>f</i> , <i>curly</i> -2 to SJ1727 <i>fla</i> 373 <i>Hr</i> - <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₃ <i>t</i>		
	' <i>g</i> ₃ <i>g</i> ₄ <i>f</i> ' ' <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₃ <i>t</i> ' ' <i>g</i> ₁ <i>g</i> ₃ <i>t</i> ' ' <i>g</i> ₃ <i>g</i> ₄ <i>t</i> '	1 and 5 1 and 2 1 and 3 1 and 4
(B) Transduction from SJ1892 <i>Hr</i> - <i>g</i> ₃ <i>g</i> ₄ <i>f</i> , <i>curly</i> -2 to SJ1632 <i>fla</i> III251 <i>Hr</i> - <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₄ <i>g</i> ₅ <i>m</i>		
	' <i>g</i> ₃ <i>g</i> ₄ <i>f</i> ' ' <i>g</i> ₁ <i>g</i> ₂ <i>g</i> ₄ <i>g</i> ₅ <i>m</i> '	1 and 3 1 and 2

Summing up the results of these two crosses and the previous report (Yamaguchi & Iino, 1969), the following order is obtained:

$$\begin{array}{c}
 \text{Hr} \\
 \text{---flaIII---flaAI---}\overbrace{\text{g}_2\text{g}_1\text{g}_4\text{---}(\text{g}_3\text{g}_5\text{f,m,t})\text{---}}^{\text{Hr}}\text{---curly-2---flaL---}
 \end{array}$$

As *curly*-2 was allocated to segment 15, these results show that all the antigenic specificity-determining areas are distributed on segments 15 and/or 16.

Fig. 4. Summarized map of *flaAI-HI-flaL* region.

DISCUSSION

The results obtained here are summarized in Fig. 4. As shown in the map, about 70 % of non-flagellate *HI* and all the *ahl*⁻ mutational sites were located in the quarter of the *HI* gene at its *flaL* side, while curly mutational sites and antigenic specificity-determining areas were located in the remaining three quarters.

The *HI* mutants studied in the present experiments are presumed to have defects in the synthesis of intact phase-I flagellin which can polymerize into flagellar filaments. It was demonstrated here that about 70 % of non-flagellate mutational sites are concentrated in the *flaL*-side quarter of *HI*. Further, this region was shown to cover none of the antigenic specificity-determining area. These results imply that the region corresponds to a section of the polypeptide chain occupying an inner part of the tertiary structure of flagellin and playing an important role in the conformation and/or polymerization of the molecule. The antigenic specificity-determining areas, allocated on the three quarters of the *HI* gene region at its *flaAI* side, may correspond to a part of the flagellin polypeptide exposed on the surface of flagellin molecule. The relatively rare detection of non-flagellate mutations in this region may mean that conformational alteration of flagellin caused by a mutational change in its surface is usually not so severe as to result in the loss of ability to polymerize. The above results and considerations conform with those of Parish, Wistar & Ada (1969), who demonstrated by chemical and immunological analyses on fg-type flagellin that all the antigenic specificities reside in the central region of flagellin polypeptide, and thus inferred that this region forms the portion of a flagellar molecule exposed on the surface of a flagellar filament. In the present study, however, the presence of an antigenically-inert area to the *flaAI* side of *HI* was not detected.

It has been reported that, not only in *Salmonella* but also in *Bacillus subtilis*, flagellar-shape mutants are not accompanied by any change in serological alteration (Iino, 1962; Iino & Mitani, 1967; Martinez *et al.* 1968). Therefore, the key region in flagellin in determining flagellar shape has been assumed to be distinct from those determining antigenic specificities. The present results confirm this assumption: a curly mutational site was separable from the antigenic specificity-determining areas in *HI*.

HI and *ahl* were initially defined as the phase-I antigen determinant and the activity controller of *HI* adjacent to it, respectively (Lederberg & Edwards, 1953; Iino, 1961). Later, *HI* was identified as the structural gene of phase-I flagellin, and *ahl*⁻ was thought to be either an operator-negative state or nonsense chain-terminating mutations in *HI* (Iino, 1969). From the present experiments, it is now known that not all the *HI* region is responsible for determining the antigenic specificities of flagellin, and the same phenotype as *ahl*⁻, i.e. defective phase-I flagella formation, can occur by a mutation in at least a

quarter of *H1* covering none of the antigenic specificity-determining areas. To avoid confusion between the symbols *H1* and *ah1*, we propose to define *ah1* hereafter as the operator region of *H1*, by analogy with the *ah2-H2-rh1* operon (Fujita, Yamaguchi & Iino, 1973; Suzuki & Iino, 1973). By this definition, the mutations previously described as '*ah1*⁻' are not *ah1*⁻ but nonsense *H1* mutations. The genuine *ah1* region is expected to be identified by isolation of mutants insensitive to the phase-I repressor.

The two-factor crosses showed the presence of intervals of some length between *H1* and *flaAI* and *H1* and *flaL*. In particular, the length of the latter interval is comparable to that of the *H1* gene. Stocker, McDonough & Ambler (1961) demonstrated that *nml*, a gene controlling the methylation of lysine residues of flagellin, is closely linked to the *H1* gene. Recently, we have assigned the position of *nml* to the space between *H1* and *flaL* (unpublished).

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