Genetic Analysis of *H2*, the Structural Gene for Phase-2 Flagellin in *Salmonella*

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Non-flagellate *H2* mutants were isolated from a phase-2 stable strain, SJW806 *H1-gt⁻ H2-enx^{on} vh2⁻*, a derivative of *Salmonella typhimurium*. By transductional crosses a deletion map and a recombination map of the *H2* gene were made. There are three regions especially rich in non-flagellate mutational sites. By the use of the deletion map, mutational sites of 21 flagellar shape mutants were also determined. Most of them were located at two regions which coincide with two of the three regions rich in non-flagellate mutational sites.

A gene, *vh2*, is closely linked to the promoter side of the *H2* gene. Three-factor transductional crosses showed that the *vh2* gene was on the left of the *H2* gene in the present map.

The *H2* gene forms part of an operon with the distal gene *rh1* which specifies the *H1* repressor. Thus, a polarity effect of the *H2* mutations on the expression of the *rh1* gene was examined by observing whether a wild-type *H1* allele introduced into the *H2* mutants was expressed or not. Many of the *H2* mutations were polar, and most of the strongly polar mutations were located in the left (promoter-proximal) half of the *H2* gene, while most of the mutations in the right half of the gene were weakly polar or non-polar.

INTRODUCTION

Most *Salmonella* species carry two structural genes, *H1* and *H2*, for flagellin, the protein component of flagellar filaments. *H1* and *H2* were originally defined as the genes determining antigenic specificity of phase-1 and phase-2 flagella, respectively. They determine not only the antigenic specificity of flagella but also the shape of flagellar filaments and the sensitivity of bacteria to flagellotropic phage (Iino, 1977).

The genes *H1* and *H2* are located far apart from each other on the *Salmonella* chromosome: *H1* at 41 min and *H2* at approximately 58 min (Sanderson & Hartman, 1978). They are expressed alternatively by the mechanism known as phase variation (Iino, 1977). The cells expressing *H1* or *H2* are said to be in phase-1 or phase-2, respectively. The *H2* gene forms part of an operon with a gene termed *rh1*, which specifies a substance repressing the expression of the *H1* gene in phase-2 cells (Fujita *et al.*, 1973). Simon and his colleagues (Zieg *et al.*, 1977; Silverman *et al.*, 1979; Zieg & Simon, 1980) demonstrated that the expression of the *H2* operon is regulated by a recombinational event which inverts a specific 900-bp DNA sequence containing the promoter region of the *H2*-operon. Furthermore, they demonstrated that the invertible region also contained a gene, *hin* (Zieg & Simon, 1980). The gene *hin* is identical with the gene *vh2*, which was originally defined as the gene controlling the frequency of phase variation (Iino, 1961) and is now known to be the gene specifying a cytoplasmic factor which catalyses the inversion of the invertible region (Kutsukake & Iino, 1980).

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A deletion map of the *H1* gene has already been reported, and some regions determining antigenic specificities of flagella as well as some mutational sites responsible for genetic alterations of flagellar shape were mapped (Horiguchi *et al.*, 1975). The genes and regions concerning phase variation make a cluster around *H2*. Little genetical analysis of the *H2* gene and its vicinity has been carried out.

In this study, a deletion map and a recombination map were constructed with non-flagellate *H2* mutants isolated from a phase-2 stable derivative of *Salmonella typhimurium*, and mutational sites of some flagellar shape mutants were mapped. The position of the *vh2* gene with respect to the present *H2* map and the polar effect of the non-flagellate *H2* mutations on the expression of the *rhl* gene were also examined.

METHODS

Organisms and media. The bacterial strains used are listed in Table 1. The flagellotropic phage χ was used to select non-flagellate mutants. Phage P22 was used as the mediator in transduction experiments. Lysates of both phages were prepared by the soft agar layer method and titered on *Salmonella* strain SJW806. The compositions of nutrient broth, nutrient agar and semisolid medium were described by Yamaguchi *et al.* (1972).

Isolation of non-flagellate H2 mutants. Spontaneous non-flagellate mutants were isolated from a phase-2 stable *Salmonella* strain SJW806 by the combined use of the flagellotropic phage χ and semisolid medium (Yamaguchi *et al.*, 1972). The non-flagellate phenotype may be caused by mutations not only in the *H2* gene but also in any of the *fla* genes. To identify *H2* mutants, non-flagellate mutants were subjected to a complementation test with *Salmonella* strain SJ814 (*H1-i⁻ H2-1-2^{straight} vh2⁺*). SJ814 is a diphasic strain which is non-flagellate in phase-1 and produces straight flagella in phase-2. As straight flagella cannot propel bacteria this strain is non-motile in both phases. A phase-2 culture of SJ814 was used to prepare the donor phage lysate. Non-flagellate mutants showing no complementation with SJ814 were judged to be *H2* mutants.

Isolation of flagellar shape mutants. Flagellar shape mutants were isolated principally according to Fujita *et al.* (1981). Broth cultures of the parental strain, SJW806, were left for 4–5 d at 37 °C. Dilutions of the culture were then spread on semisolid medium. After overnight incubation, clones which formed compact and dense colonies were examined for their motility in liquid medium with a photomicroscope. Clones showing behaviour characteristic of flagellar shape mutants (Iino, 1969; Fujita *et al.*, 1981) were further examined for their flagellar morphology with an electron microscope.

Test for complementation and recombination. Complementation and recombination between pairs of non-motile mutants (including flagellar shape mutants) were examined by P22-mediated transduction. The transduction mixture was streaked in lines on semisolid medium. Production of trails (abortive transductants) and swarms (complete transductants) in the medium were used as the criteria for complementation and recombination, respectively (Yamaguchi *et al.*, 1972). When 1.0 ml of the mixture of equal volumes of the donor phage suspension (1.0×10^{10} p.f.u. ml⁻¹) and the recipient bacterial suspension (1.0×10^9 cells ml⁻¹) did not produce a swarm, the mutational sites of the donor and the recipient were regarded as overlapping each other.

For two-factor crosses between *H2* mutants, the donor phage suspension adjusted to 1.0×10^{10} p.f.u. ml⁻¹, was mixed with the same volume of the recipient bacterial suspension containing 1.0×10^9 cells ml⁻¹. After 10 min adsorption, 0.5 ml of the mixture was streaked in lines on semisolid medium. After overnight incubation, the number of swarms (motile recombinants) was counted. The recombination frequency between donor and recipient mutational sites was expressed as a percentage of the number of swarms obtained in the control transduction in which the *H2⁺* strain SJW806 was used with the same recipient. In each control transduction, more than 2000 swarms were always counted.

Observation of flagellar shape by electron microscopy. Bacteria were grown in broth overnight without shaking, harvested by low-speed centrifugation and resuspended in distilled water containing 5 % formaldehyde. Samples were negatively stained with phosphotungstic acid at pH 6.8 and examined in a JEM T7 electron microscope.

Table 1. *Bacteria*

Strain no.	Genotype	Characteristics
SJW806	<i>H1-gt⁻ H2-enx^{on} vh2⁻</i>	A phase-2 stable derivative of <i>Salmonella typhimurium</i> . <i>H1-gt</i> was transduced from strain SJ925 (Yamaguchi <i>et al.</i> , 1972), and <i>H2-enx^{on}</i> and <i>vh2⁻</i> from <i>S. abortusequi</i> SL23 (Iino, 1961).
SJ814	<i>H1-i⁻ H2-1-2^{straight} vh2⁺</i>	An O-H variant of <i>S. typhimurium</i> producing straight flagella in phase-2 (Iino, 1969).
SJW1103	<i>H1-i H2-enx^{-off} vh2⁻</i>	A phase-1 stable derivative of <i>Salmonella typhimurium</i> . <i>H2-enx^{-off}</i> and <i>vh2⁻</i> were introduced from SJW916, an <i>H2⁻</i> mutant of SJW806.

Preparation of the anti-*i* flagella serum. Cells of SJW1103 (the antigen type of flagella is 'i') were suspended in 0.5% formalinized saline to give about 1×10^8 bacteria ml^{-1} . A rabbit was immunized by five successive intravenous injections with a total of 6 ml of the bacterial suspension at 3 d intervals, and bled 1 week after the final injection. Antibodies specific to the somatic antigens were removed by absorbing the serum with a non-flagellate strain derived from SJW1103.

Genotype symbols. Alleles of *H1* and *H2* genes expressing different flagellar antigen types are indicated by symbols designating the antigen types, e.g. *H1-i*, *H2-enx*, etc. To indicate the stable states (active and inactive) of the *H2* gene in *Vh2*⁻ strains, superscript symbols 'on' and 'off' are used, e.g. *H2-enx*^{on} for the active state and *H2-enx*^{off} for the inactive state of the *H2-enx* allele according to Enomoto & Stocker (1975). The *H2* alleles which are active but carry non-flagellate mutations are described as, for example, *H2-enx*⁻. Flagellar shape mutations are indicated by the symbol in parentheses (Fs), placed after the mutation numbers, e.g. *H2-51* (Fs).

RESULTS

Isolation of non-flagellate H2 mutants

Using the flagellotropic phage χ as the selecting agent, 7650 spontaneous non-flagellate mutant clones were isolated from a phase-2 stable *Salmonella* strain, SJW806 *H1-gt*⁻ *H2-enx*^{on} *vh2*⁻. Among them, 253 clones were identified as *H2* mutants by the complementation test with the phase-2 culture of *Salmonella* strain SJ814 *H1-i*⁻ *H2-1-2*^{straight} *vh2*⁺. Among the *H2* mutants, 78 clones were stable and the others revertible.

Mapping of the H2 gene

With all the stable *H2* mutants and 86 revertible ones whose reversion frequencies were not too high, deletion mapping of the *H2* gene was carried out by the following two series of transductional crosses. Transductions were first carried out in all pairwise combinations among the stable mutants, and then from revertible mutants to stable ones. Of 78 stable mutants, 33 were shown to be multisite or deletion mutants. From their overlapping pattern, the *H2* gene was divided into 31 deletion segments as shown in Fig. 1. Segments were given numbers from no. 1 to 31 running from left to right. Mutational sites of the mutants that were stable but yielded wild-type recombinants with all the revertible mutants were included in the group of single site mutations in the map.

As seen in Fig. 1, the deletion mapping could not determine the orientation of two regions (segments no. 1 to no. 4 and no. 12 to no. 20). Nor could deletion mapping determine the positions of the single site mutations finally allocated to segments no. 5, 11 and 21. Therefore, recombination frequencies between a standard mutational site and single site mutations were examined. As the standard site, the mutational site of strain SJW831 *H2-120* (which had been allocated to segment no. 6 by deletion mapping) was used. Transduction was carried out from SJW831 and the parental *H2*⁺ strain SJW806 to the mutants to be examined, and recombination frequencies were calculated as described in the Methods. Selected results are given in Table 2. They show that the order of the segments flanking segment no. 6 is 1 to 4 – 6 – 12 to 20. The results also permit the mapping of single site mutations to one of the segments no. 5, 11 or 21 as shown in Fig. 1.

In the deletion map (Fig. 1), the segments are arbitrarily shown to be of equal length. To know more precisely the distribution of non-flagellate mutational sites within the *H2* gene, mapping of single site mutations by recombination frequencies was carried out. The mutational site of strain SJW913 *H2-200*, the leftmost mutational site in the *H2* gene, was used as the standard site. Transduction was carried out from SJW913 and the *H2*⁺ strain SJW806 to all the single site mutants shown in Fig. 1, and recombination frequencies were calculated. Figure 2 shows the distribution of the mutational sites according to their map positions, made proportional to recombination frequencies. Ranges of distribution of mutational sites assigned to respective deletion segments are also shown in the figure. Discrepancies found in the order of some mutational sites between the deletion map and the recombination map are within the limit of errors of recombination frequencies. As seen in the figure, the distribution of mutational sites is not random. Three regions, that is, regions around the map positions 4.0, 12.5 and 21.5, respectively, are especially rich in non-flagellate mutational sites.

Fig. 1. Deletion map of the H2 gene in *Salmonella* strain SJW806 H2-enz. Horizontal lines represent the extent of respective deletions. Mutations that were stable but yielded flagellate recombinants with all the revertible mutants were included in the group of single site mutations. Orientation of two regions comprising segments no. 1 to no. 4 and no. 12 to no. 20, respectively, and positions of single site mutations assigned at segments no. 5, no. 11 and no. 21 were determined by the help of recombination frequencies.

Table 2. *Recombination frequencies between strain SJW831 H2-120 and non-flagellate H2 mutants*

Recombination frequencies were measured by transduction. A known volume of the transduction mixture was brushed in lines on semisolid medium. After overnight incubation, the numbers of swarms were counted. The recombination frequency was expressed as percentage of the number of swarms obtained in transduction from the wild-type strain SJW806.

Recipient			No. of motile recombinants in transduction from		Recombination frequency (B/A, %)
Strain	Mutation	Segment*	(A) SJW806 <i>H2</i> ⁺	(B) SJW831 <i>H2</i> -120	
SJW913	<i>H2</i> -200	1	2114	120	5.7
SJW869	<i>H2</i> -157	2	3019	136	4.5
SJW928	<i>H2</i> -215	3	2623	105	4.0
SJW925	<i>H2</i> -212	4	2460	45	1.8
SJW986	<i>H2</i> -263	5	2011	33	1.6
SJW1061	<i>H2</i> -338	5	2338	29	1.2
SJW1007	<i>H2</i> -284	5	2016	25	1.2
SJW831	<i>H2</i> -120	6	2320	0	0.0
SJW810	<i>H2</i> -101	11	2784	23	0.8
SJW944	<i>H2</i> -230	12	2439	35	1.4
SJW818	<i>H2</i> -109	13	2428	49	2.0
SJW812	<i>H2</i> -103	18	2954	77	2.6
SJW843	<i>H2</i> -131	19	2783	162	5.8
SJW895	<i>H2</i> -182	20	2856	172	6.0
SJW824	<i>H2</i> -113	21	2291	161	7.0
SJW837	<i>H2</i> -125	21	2401	168	7.0
SJW838	<i>H2</i> -126	21	2235	157	7.0
SJW841	<i>H2</i> -129	21	2512	189	7.5

* Segment no. in the deletion map of the *H2* gene (Fig. 1), to which respective mutational sites were assigned.

Fig. 2. Distribution of non-flagellate mutations in the *H2* gene. The scale represents the distance from the leftmost mutational site *H2*-200, made proportional to recombination frequencies. Mutational sites shown by closed circles are piled up every 0.25 interval. Circled figures represent the segment numbers in the deletion map (Fig. 1). The ranges shown by the segment numbers represents the distribution of mutational sites assigned to respective segments in the deletion map.

Mapping of the mutational sites responsible for alteration of flagellar shape

Bacterial flagella show a helix with wavelength and wave height characteristic of bacterial strains; e.g. the wavelength and the wave height of flagella of strain SJW806 are $2.47 \pm 0.08 \mu\text{m}$ and $0.32 \pm 0.02 \mu\text{m}$, respectively. Several types of flagellar shape mutants have been reported. Curly mutants carry flagella of the wavelength about half the normal. Flagella of straight mutants lack the wave form. Polymorphous mutants carry flagella of several distinct forms including straight, curly and sometimes normal. Heteromorphous mutants carry irregular-

shaped flagella. These genetic alterations in flagellar shape are due to mutations in the flagellin structural gene, *H1* or *H2* (Iino, 1969, 1977). To understand the genetic determination of flagellar shape it is desirable to know the correlation between altered flagellar shapes and the sites of mutations responsible for the alterations. Therefore phase-2 flagellar shape mutants were isolated and their mutational sites on the present deletion map determined.

From strain SJW806, 150 spontaneous flagellar shape mutants were isolated by the procedure described in the Methods. Twenty mutants, randomly chosen, were scrutinized for their flagellar shape by electron microscopy, and classified into four groups. The first group (curly type) comprised SJW548 *H2-52* (Fs) and nine other mutants; average wavelength and wave height of their flagella were $1.34 \pm 0.03 \mu\text{m}$ and $0.29 \pm 0.01 \mu\text{m}$, respectively. The second group (small amplitude type) comprised SJW540 *H2-54* (Fs) and six other mutants; the average wavelength and wave height of their flagella were $0.45 \pm 0.02 \mu\text{m}$ and $0.100 \pm 0.004 \mu\text{m}$, respectively. In liquid medium, mutants of these two groups showed rotational movement and aggregation that are characteristic of curly mutants (Iino, 1969). The third group comprised only one polymorphous mutant, SJW550 *H2-64* (Fs). Most flagella of this strain were either normal or curly, and a few (less than 5 % of flagella) carried both types of waves. In liquid medium, most organisms of this strain moved rotationally, but some wriggled and a few showed normal translational movement. Aggregation in liquid medium was often observed but was weaker than that of the two former groups. The fourth group comprised two heteromorphous mutants, SJW542 *H2-56* (Fs) and SJW544 *H2-58* (Fs). Their flagella were mostly irregular with wavelength longer than normal. Aggregation in liquid medium was not observed in these mutants. An electron microscope survey of the remaining 130 flagellar shape mutants showed that they belonged to or closely resembled one or other of the four groups. No straight mutant was found among them.

Using the *H2* deletion mutants as recipients, the mutational sites of the twenty flagellar shape mutants were mapped by transductional crosses. As no straight mutant was obtained from strain SJW806, the phase-2 straight strain SJ814 was included in the experiment. Results are shown in Fig. 3. The mutational sites were distributed roughly to two regions, segments no. 6 to no. 9 and segments no. 25 to no. 30. These regions almost coincide with two of the three regions that are especially rich in non-flagellate mutational sites.

*Position of the $vh2$ gene relative to the *H2* map – determination of the promoter side of the *H2* map*

No mutant has so far been obtained which has been confirmed to be defective in the promoter of the *H2* operon. However the promoter region of the *H2* operon is in the invertible region or so-called phase determinant region (PD) (Silverman *et al.*, 1979; Zieg & Simon, 1980; Enomoto & Stocker, 1975). Moreover the gene termed *hin* or *vh2* is included in the PD region (Zieg & Simon, 1980). The *vh2* gene promotes phase variation by specifying the cytoplasmic factor that catalyses the inversion of the PD region (Kutsukake & Iino, 1980). Strains carrying a *vh2*⁻ mutation, in which the *H2* operon has been stabilized in either the 'on' or the 'off' state, are available (Iino, 1961). Thus, an attempt was made to determine the position of a *vh2*⁻ mutation relative to the *H2* gene to discover the location of the promoter on the *H2* map.

Determination of the position of the *vh2* gene relative to the *H2* gene was accomplished by three-factor P22 transductional crosses between a *Vh2*⁺ strain and phase-2 stable *Vh2*⁻ strains both carrying a non-motile mutation in the *H2* gene. Motility was used as the selected marker and the *Vh2* character as the unselected marker. The *Vh2*⁺ strain used was SJ814 *H1-i*⁻ *H2-enx*^{straight} *vh2*⁺. This strain shows phase variation between non-flagellate phase-1 and straight flagellar, therefore non-motile, phase-2. The mutational site responsible for phase-2 straight flagella in SJ814 is located at deletion segment no. 29 (Fig. 3) as described above. The *Vh2*⁻ strains used were four non-flagellate *H2* mutants *H1-gt*⁻ *H2-enx*^{-on} *vh2*⁻ carrying deletions in the *H2* gene; that is, SJW842 *H2-130*, SJW1039 *H2-316*, SJW1041 *H2-318* and SJW1002 *H2-279*. Deletions in the first three strains are to the left of the straight flagellar mutational site of SJ814 and that in the fourth is to the right of it. The latent phase-1 in these strains is non-flagellate because of the *H1-gt*⁻ mutation.

Fig. 3. Distribution of flagellar shape mutations in the deletion map of the *H2* gene. Flagellar shape mutants were isolated from *Salmonella* strain SJW806 *H2-enx*, except for the straight flagellar mutant SJ814 *H2-1-2^{straight}* whose mutational site is shown by *1-2^{straight}* in this figure. Circled figures represent the segment numbers in the deletion map (Fig. 1).

Table 3. Determination of the position of the *vh2* gene with respect to the *H2* gene by three-factor transductional crosses

Three-factor transductional crosses were carried out from the phase-2 culture of *Salmonella* strain SJ814 *H1-i⁻ H2-1-2^{straight} vh2⁺* to four phase-2 stable *H2⁻* strains *H1-gt⁻ H2-enx⁻ on vh2⁻*. *H2⁺* recombinant swarms on semisolid medium were isolated and their *Vh2* character was determined. The relative order of the mutational sites, inferred from these results, is shown in Fig. 4. The figures in parentheses represent crossover regions shown in Fig. 4.

Transduction recipient	No. of motile <i>H2⁺</i> recombinants carrying the allele	
	<i>vh2⁺</i>	<i>vh2⁻</i>
SJW842 <i>H2-130</i>	122 (1,3)	12 (2,3)
SJW1039 <i>H2-316</i>	143 (1,3)	0 (2,3)
SJW1041 <i>H2-318</i>	153 (1,3)	0 (2,3)
SJW1002 <i>H2-279</i>	0 (1,2,3,4)	120 (4,5)

Transduction was carried out from a phase-2 culture of SJ814 to the nonflagellate *H2* mutants. Transduction mixtures were streaked in lines on semisolid medium and motile *H2⁺* recombinants that appeared as swarms were then examined for their *Vh2* character using the following principle. The *H2⁺* recombinants that have received the *vh2⁺* allele from the donor will segregate non-flagellate phase-1 cells expressing *H1-gt⁻* in their descendants through phase variation. On the other hand the *H2⁺* recombinants that have retained the recipient *vh2⁻* allele will produce motile phase-2 cells only. Thus they can be distinguished from each other by spreading cultures of them on semisolid medium. The *H2⁺* *Vh2⁺* clones will produce not only swarming colonies of motile phase-2 cells but also compact colonies of non-flagellate phase-1 cells, while the *H2⁺* *Vh2⁻* clones will produce swarming colonies of motile phase-2 cells only.

Results of the three-factor transductional crosses are shown in Table 3. When SJW1002 *H2-279* was the recipient, all the motile *H2⁺* recombinants retained the recipient *Vh2⁻* character. When the other three deletion mutants were the recipients, all or almost all the motile *H2⁺* recombinants had acquired the donor *Vh2⁺* character. Assuming that each of these motile recombinants was produced by two crossovers, these results can be explained only if the *vh2* gene is located to the left of the *H2* gene, as shown in Fig. 4. In crosses in which SJW1039 *H2-316* and SJW1041 *H2-318* were used as recipients, all the *H2⁺* recombinants were *Vh2⁻*, suggesting that the deletions in these strains cover the *vh2⁻* mutational site.

Fig. 4. An arrangement of the $vh2$ and $H2$ mutational sites, that explains the occurrence of the $H2^+$ recombinants in three-factor crosses shown in Table 3. The horizontal lines represent the deleted regions in recipients. According to this arrangement, the occurrence of all the $H2^+$ recombinants shown in Table 3 is explained by two crossovers. The deletions $H2-316$ and $H2-318$ may extend to the position of the $vh2$ mutation.

Fig. 5. Relation between motility and total length of phase-1 flagella per cell in $H2$ mutants in which the transduced $H1-i$ allele is expressed to various degrees. Small inocula of the $H2$ mutants carrying the transduced $H1-i$ allele were placed on semisolid medium plates. After 8 h incubation, the areas of swarms were compared with that of the phase-1 stable strain SJW1103, the donor of the $H1-i$ allele. Total length of flagella per cell is the mean of measurements on 70 to 80 cells. The result shows that the relative size of swarms is almost proportional to the total length of flagella per cell.

Polar effect of non-flagellate $H2$ mutations on the expression of the $rh1$ gene

The $H2$ gene constitutes an operon together with the $rh1$ gene that codes for a cytoplasmic substance repressing the expression of the $H1$ gene in phase-2 cells (Fujita *et al.*, 1973). Silverman *et al.* (1979) obtained results showing that in the operon the order of transcription is $H2$ and then $rh1$. Thus, it is predicted that some of the non-flagellate $H2$ mutants should have a polar effect on the expression of the $rh1$ gene. To test this, a wild-type $H1$ allele was introduced by transduction into the non-flagellate $H2$ mutants $H1-gt^- H2-enx^- \cdot on\ vh2^- rh1^+$ in place of the preexisting $H1-gt^-$ allele. If an $H2$ mutation has a polar effect on the $rh1$ gene, the wild-type $H1$ allele introduced into it will be expressed. Transductant clones that have received the wild-type $H1$ allele will appear as swarms in semisolid medium.

Transduction of the wild-type $H1$ allele was carried out from a phase-1 stable strain, SJW1103 $H1-i H2-enx^- \cdot off\ vh2^-$, to all the single site non-flagellate $H2$ mutants. The transduction mixture was streaked in lines on semisolid medium and incubated overnight. As expected, swarms appeared in transductions with many but not all of the recipients. The size of swarms varied with recipients. Swarmed clones were subcultured on nutrient agar and examined for their flagellar antigen type by the slide agglutination test with anti- i serum. All of them were agglutinated by the antiserum, showing that in these swarmed clones the introduced $H1-i$ allele was expressed.

Fig. 6. Polarity values of H2 mutations on the expression of *rhI*, plotted according to their location in the H2 gene. Polarity values were expressed as the relative size of phase-1 swarms to that of *Salmonella typhimurium* SJW1103. The abscissa represents the distance from the leftmost mutation H2-200, made proportional to recombination frequencies.

The number of flagella per cell and the length of individual flagella were examined by electron microscopy with representative transductant clones differing from each other in size of swarms. In general, the larger the spreading ability of a clone the larger both the number of flagella per cell and the average length of flagella. Furthermore the average values of the total length of flagella per cell were roughly proportional to the swarmed area of the clones in semisolid medium, as seen in Fig. 5. Assuming that the total length of flagella per cell is proportional to the production of flagellin by the cell and that the production of flagellin is inversely proportional to the amount of the *H1*-repressor produced by the *rh1* gene, we tentatively define here the size of the swarm of a transductant clone relative to that of the wild-type strain SJW1103 as the polarity value of its *H2* mutation on the *rh1* gene. Thus, a polarity value of 1 indicates that the *H2* mutation in the clone is strongly polar, whereas a polarity value of 0 indicates that the *H2* mutation is non-polar. Mutants that did not produce any phase-1 swarm when the wild-type *H1-i* allele was introduced were regarded as non-polar. In Fig. 6, polarity values calculated for single site *H2* mutations were plotted according to their location on the recombination map of the *H2* gene. From the figure, it is immediately apparent that many strongly polar mutations are located in the left half of the *H2* gene, while most of the mutations located in the right half are weakly polar or non-polar.

The *H2* deletions shown in Fig. 1 were also examined for polarity on the *rh1* gene. Among them, eight relatively short deletions, that is *H2-124* (SJW836), *H2-134* (SJW846), *H2-136* (SJW848), *H2-148* (SJW860), *H2-274* (SJW997), *H2-275* (SJW998), *H2-279* (SJW1002) and *H2-308* (SJW1031), were shown to be non-polar, while the other 27 deletions were strongly polar.

DISCUSSION

Among the non-flagellate mutants those lacking the flagellar filament but retaining other parts of flagella, a class to which the non-flagellate *H2* mutants belong, are sensitive to the flagellotropic phage χ (Yamaguchi *et al.*, 1977) but their sensitivity to χ is rather low in liquid medium. Thus, non-flagellate *H2* mutants were selected efficiently by the use of the phage χ in semisolid medium.

The genes and regions concerning phase variation cluster around the *H2* gene, but genetical analysis of the *H2* gene has lagged behind that on the *H1* gene. The deletion map of the *H2* gene constructed here will facilitate the genetical analysis of the *H2* gene and its vicinity.

Mapping of non-flagellate *H2* mutations by recombination frequencies (Fig. 2) showed that there are three especially mutable regions in the *H2* gene. Lowy & Hanson (1965) proposed a model for flagellar filaments constructed of spherical flagellin molecules of 5.6 nm diameter. Subsequently an elongated shape was proposed for the flagellin molecule to explain the chevron appearance of filaments observed by electron microscopy (O'Brien & Bennet, 1972). Shirakihara & Wakabayashi (1979) showed by three-dimensional image reconstruction from electron micrographs that in flagellar filaments flagellin molecules are highly elongated and have a beaded substructure, that is, there are four high density regions or domains C, I, M and S, situated at radii of about 1.5, 4.0, 7.0 and 8.0 nm in flagellar filaments. According to their model, the inner regions of flagellin, domains C and I and a part of the domain M, may contribute to the interaction with neighbouring flagellin molecules. The domain S and the major part of the domain M protrude at the surface of the filaments. The three regions in the *H2* gene which were shown to be especially rich in non-flagellate mutational sites may correspond to the inner high density regions which are important for flagellin to polymerize into flagellar filaments.

The mutational sites of three phase-1 curly flagellar strains are located in a single segment of a sixteen-segment deletion map of the *H1* gene (Horiguchi *et al.*, 1975). We show here that the mutational sites of 21 phase-2 flagellar shape mutants are located in two separate regions. The regions almost coincide with two of the three regions that are rich in non-flagellate mutational sites. This result shows that these regions are important not only for assembly of flagellin into a filament but also for the determination of the mode of assembly.

Among mutants with altered flagellar antigen, isolated from a phase-1 stable *Salmonella* strain, we have recently found a mutant in which the shape of flagellar filaments is normal but the molecular weight of flagellin has been reduced to three-quarters that of the parental type

flagellin (S. Yamaguchi, unpublished). Genetical analysis showed that in the *H1* gene of the mutant a region had been deleted, where non-flagellate mutational sites are rarely located. The three-dimensional image reconstruction from electron micrographs of its flagellar filament revealed that in flagellin the size of the high density regions S and M was diminished (Y. Shirakihara *et al.*, unpublished). These observations suggest that the region deleted in this mutant participated in determining the antigenic specificity of flagellin but not in the polymerization or determination of shape of flagellar filaments. Thus, the combination of three-dimensional analysis and genetical analysis of flagellins carrying various mutant characters is expected to contribute much to the understanding of the relation between the structure and the function of flagellin.

The non-flagellate *H2* mutants obtained here may include mutants defective in the promoter of the *H2* operon, but they cannot be distinguished from those defective in the *H2* structural gene. Therefore no information on the direction of transcription can be obtained solely from the present map of the *H2* gene. Fortunately the promoter of the *H2* operon has been mapped between the *H2* gene and the *hin* or *vh2* gene (Zieg & Simon, 1980). Thus, to determine which end of the *H2* map is promoter-proximal, the position of the *vh2* gene relative to the *H2* gene was examined. The *vh2* gene was shown to be located to the left of the *H2* gene, so that the promoter is located at the left end of the *H2* gene on the present map.

Many of the non-flagellate *H2* mutations have a polar effect on the expression of the *rh1* gene. The polarity value of an *H2* mutant is defined as the relative size of a swarm of the *H2* mutant in semisolid medium, after introduction of a wild-type *H1* allele. This definition is based on the observation that the relative size of swarms of the transductant clones was roughly proportional to the total length of flagella per cell. We have no means at present to measure the amount of *H1* repressor substance directly. In general, the further to the right within the *H2* gene the mutational site, the lower the polarity value of the mutation. This indicates that the *rh1* gene is located to the right of the *H2* gene, so that the promoter of the *H2* operon must be located to the left of the *H2* gene.

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