Recombination in $H_1$, the Gene Determining the Flagellar Antigen-i of *Salmonella typhimurium*; Mapping of $H_1$ and fla Mutations

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(Accepted for publication 27 June 1969)

**SUMMARY**

Five *fla* mutations of *Salmonella typhimurium* LT2 have been mapped by transduction. They were isolated from five motile (*fla*) strains each with a different form of antigen-i, caused by mutation of $H_1$, the structural gene for phase-1 flagellin. $H_1$ was cotransduced (frequency 0.1 to 0.5) with each of the *fla* mutations. Three-point crosses using a serological selection technique indicate the following order: *fla*-50-*fla*-58-*fla*-55-$H_1$. The position of *fla*-52 was not discovered. If the *fla* complementation groups correspond to the *fla* genes their order is *fla*-*fla*-$H_1$.

Of the five $H_1$ mutations four (M5, M20, M11, M12) appear to be very closely linked. The fifth (M6) is outside this cluster. Transductants with functionally and antigenically normal antigen-i were obtained, at a very low frequency, in crosses between *i*-curly and *fla* strains: they are attributed to crossing over within $H_1$ between the 'curly' mutation and the mutations causing alteration in serological character. Some earlier conclusions (Joys & Stocker, 1963) as to gene order, especially within $H_1$, now seem unjustified.

**INTRODUCTION**

The numerous phase-1 antigens of the Salmonella group are determined by different forms of a gene termed $H_1$ (Lederberg & Edwards, 1953). We have described several altered forms of antigen-i, the wild-type phase-1 antigen of *Salmonella typhimurium* strain LT2, obtained by selection of mutants, termed M, able to spread faster than the parent strain through semisolid medium containing enough anti-i serum to retard its spreading growth (Joys & Stocker, 1966). Their alterations in serological character presumably result from local changes, probably single amino acid substitutions, in some antigenically active part of the flagellar protein (flagellin); indeed, alterations in the position of a peptide have been detected in the peptide ‘maps’ of digests of four of the mutant *i* flagellins (McDonough, 1962). It seemed probable that each mutant resulted from alteration of the $H_1$ gene at only a single point. We here report an attempt to map these mutations in some of the serological mutants.

Non-flagellate mutants, resulting from mutations in *fla* genes closely linked to $H_1$, and therefore co-transducible with it, were obtained from each serological mutant,

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so that the fla mutations might serve as external markers in crosses between $HI$ mutants; for the present experiments five such fla mutants, each obtained from a different serological mutant, were chosen from amongst 11 whose isolation and complementation behaviour we have described (Joys & Stocker, 1965). In transductional crosses of some pairs of serological mutants we readily showed recombination within the $HI$ gene leading to the production of the ancestral (wild type) gene and antigen. We could not draw any firm inferences about the order of mutations within $HI$ and the present more complete investigation does not confirm some earlier tentative conclusions (Joys & Stocker, 1963). We have been able, however, to determine the order of several fla genes relative to each other and to $HI$.

Iino (1962) described a strain of Salmonella typhimurium which when in phase-1 produced functionally deficient flagella with an abnormally short wavelength ('curly' phenotype). He inferred that the deficiency was due to a mutation which in his crosses could not be separated from $HI$; it now seems probable that this mutation is within the structural gene for phase-1 flagellin. We crossed this mutant with fla derivatives of serological mutants with the object of defining more precisely the position of the 'curly' mutation.

**METHODS**

**Bacterial strains.** Five mutants with altered forms of antigen $i$ and one stable fla derivative of each were used: $M_5$ fla$^+$ and fla-50; $M_6$ fla$^+$ and fla-52; $M_10$ fla$^+$ and fla-57; $M_{11}$ fla$^+$ and fla-55; $M_{12}$ fla$^+$ and fla-58 (Joys & Stocker, 1965, 1966). A spontaneous mutant resistant to high concentrations of streptomycin was selected in each fla line. Genetically labelled sublines of the fla$^+$ strains, for use as donors in transduction experiments and as absorbing antigens in the preparation of specific antisera, were obtained by the isolation of (non-lysogenic) pur$^+$ and pur$^+$ pro$^+$ forms by successive transduction with phage P22. H4 (Yura, 1956). The phase-1-curla strain used was Salmonella typhimurium sw 577 (Iino, 1962). All the above strains make flagellins containing N-methyllysine (Stocker, McDonough & Ambler, 1961). S. typhimurium strain $s_{D_7}$, used in some experiments as fla$^+$ donor with a phase-1 antigen unrelated to $i$, is a (P 22-sensitive) $LT_2$ transductant in which the S. typhimurium wild-type $HI$ gene has been replaced by the $HI$ gene of S. abony, determining antigen-b (Spicer & Datta, 1959). The flagellins of this strain lack N-methyllysine, because of co-transduction of the nml gene of the donor with its $HI$ gene (Stocker, McDonough & Ambler, 1961).

**Serological methods.** Sera specific for each of the mutant forms of antigen-$i$, for use in selection in semisolid medium, were prepared by absorption of antimutant sera with pur$^+$ pro$^+$ (streptomycin-sensitive) bacteria having the wild-type antigen-$i$ (Joys & Stocker, 1966) and sterilized by Seitz filtration. Sera specific for the wild-type form of antigen-$i$ were prepared by the absorption of anti-$i$ (wild type) serum with mutant forms of the antigen (Joys & Stocker, 1966). The anti-$i$,2 serum used, active on the phase-2 antigens of all the strains, was obtained by inoculation of a rabbit with a Salmonella typhimurium strain which is non-flagellate when in phase-1 owing to an $ahI$ mutation (Iino, 1961); it was absorbed with an $i$ (wild type) suspension to remove anti-O and possible anti-$i$ activity. The concentration of an absorbed serum used in semisolid medium was that which just sufficed to prevent spreading of fla$^+$ transductants with the homologous antigen, and was about double that needed
Recombination of \( H_1 \) in *S. typhimurium* for agglutination in a micro test (Joys & Stocker, 1966). Tests on the flagellar antigens of clones recovered from spreading swarms confirmed the efficacy and specificity of the serum selections.

Recombinants with wild-type antigen-\( i \) were sought by selection in semisolid medium containing both serum specific for the mutant \( i \) antigen of the recipient parent and serum specific for that of the donor; the efficacy of this selective medium was tested in each series of experiments by inoculation of a plate with about 20 motile bacteria producing the wild-type antigen together with about \( 10^8 \) fla bacteria.

**Transduction methods.** Phage P22 grown on a fla+ or fla donor strain was added to a 1 ml. volume of a 37\(^\circ\) overnight shaken broth culture of a fla recipient strain (about \( 10^8 \) bacteria) to give a phage : bacterium ratio of about 10:1. After 20 min. at 37\(^\circ\), ten-fold dilutions to \( 10^{-4} \) were made and 'standard drop' inocula (0.02 ml.) of each dilution, of the undiluted mixture, and of the resuspended deposit (0.1 ml.) from the centrifuged transduction mixture, were placed on four sets of 5 cm. plates of semisolid medium. Appropriate control plates tested the bacterial sterility of sera and phage. Streptomycin (1 mg./ml.), added to the semisolid medium as a precaution against airborne contamination, would have prevented the growth of any viable donor bacteria present in the lysate, or of any viable bacteria of the absorbing strain present in the sera, since these strains were streptomycin-sensitive. One set of plates contained no serum and a second set only anti-\( i,2 \) serum. The approximately equal yields of swarms on these two sets confirmed that the majority of cells in the recipient fla culture were, as intended, in latent phase 1, so that fla+ transductants expressed their \( H_1 \) genes. The highest dilution giving swarms in these two sets gave an estimate of the total number of fla+ transductants, most of which would retain the \( H_1 \) gene of the recipient since the rate of co-transduction of \( fla \) and \( H_1 \) was \( < 0.5 \) for the fla mutants used. A third set contained anti-\( i,2 \) serum and serum specific for the mutant antigen of the recipient; the yield of swarms on these plates showed the number of fla+ transductants with the phase-\( i \) antigen of the donor, or with wild-type (recombinant) antigen. A fourth set contained anti-\( i,2 \) serum and two anti-\( i \) sera, one specific for the mutant antigen of the donor and the other specific for the mutant antigen of the recipient; swarms in these plates indicated the production of fla+ transductants with the wild-type antigen-\( i \). The absence of fla+ revertants was tested in each experiment by inoculation of semisolid medium with the recipient strain treated with broth instead of phage. In an additional control experiment each fla recipient was treated with phage grown on its fla+ parent, so that all fla+ transductants would have the same mutant antigen. The absence of swarms on plates containing serum specific for this antigen confirmed the efficacy of the serum and showed that the population of fla+ transductants did not give second-step serological mutants (in particular revertants) able to spread in its presence.

After 48 h of incubation at 37\(^\circ\), the highest dilution yielding spreading growth (i.e. confluent swarms) was recorded for each set of plates and subcultured for determination of flagellar antigens by slide agglutination. Growth from plates selective for the recombinant antigen was tested for lack of agglutination by sera specific for the donor and recipient antigens and for agglutination by anti-\( i \) (wild type) sera absorbed with the donor antigen or with the recipient antigen. Several cultures which satisfied these criteria were used to absorb an anti-\( i \) (wild type) serum, and the fully absorbed serum was tested on wild-type \( i \) agglutinable suspension. A rabbit serum was prepared
against one such recombinant, absorbed with wild-type i antigen and tested for agglutinating activity against the inoculated strain. All suspected recombinants were tested for the nutritional characters of the recipient pur pro strains.

In experiments in which the donor strain was SW577, the Salmonella typhimurium phase-1-'curly' mutant, only three sets of plates were used, one without antiserum, one with only anti-i,2 serum and the third with anti-i,2 serum and serum specific for the mutant antigen-i of the fla recipient.

RESULTS

Order of fla mutations and H1

Each fla strain, derived from a fla+ parent with an altered phase-1 antigen, was treated with phage P22 lysates of the fla+ parent and the fla derivative of each of the other four mutants with altered antigen-i, and the numbers of fla+ transductants with the recipient, donor and wild-type (recombinant) forms of antigen-i were determined. Table 1 records the results of one set of experiments. Nearly all the crosses were repeated at least once, with essentially similar results. No fla+ revertants were detected on the control plates.

Fig. 1. Mapping of fla mutations by transduction. The figure shows the three possible orders of two fla mutations with respect to a mutation, M, in H1. The upper line in each diagram represents the donor fragment. The broken lines represent the crossovers required to yield a motile M1 recombinant in each case. We expect that depending on the order of the markers a fla donor may yield far fewer motile M1 recombinants than its fla+ analogue.

Consider first the inferences which may be drawn about the order of the fla mutations relative to each other and to H1. All crosses, except those involving M6, which is discussed below, yielded very few or no wild-type i swarms. Our H1 mutations must therefore be very close together and may be treated in the genetic analysis as changes at a single site. In this way the fla mutations can be ordered with respect to H1.

A fla+ donor having a mutant H1 gene (say M1) will yield motile transductants in a cross against a fla recipient. When the recipient has a different H1 mutation (say
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The pairwise combinations of the HI mutants tested (four, since M6 and its fla derivative are excluded) provide twelve crosses, in each of which the effect of a fla mutation in the donor was tested (Table I). In crosses 2, 4, 17 and 18 (indicated A in Table I) the proportion of fla+ transductants with the donor HI gene was decreased at least a hundredfold when the donor was fla. We infer that in these crosses the donor fla is between HI and the recipient fla. The orders thus indicated are: fla-50—fla-57—HI; fla-50—fla-58—HI; fla-58—fla-57—HI; and fla-58—fla-55—HI. Taken together these give: fla-50—fla-58—(fla-57, fla-55)—HI. In crosses 3 and 10 (indicated B in Table I), each of which was repeated at least twice, the estimated proportion of donor-type swarms was consistently decreased when the donor was fla, but only by a factor of ten. If this decrease results from the need for four crossovers the orders indicated are: fla-57—fla-55—HI and fla-50—fla-55—HI, both compatible with the order already inferred and combining with it to give: fla-50—fla-58—fla-57—fla-55—HI. In the other six crosses (indicated C in Table I), which are the reverse crosses of the six so far considered, the fla mutation of the donor had no detectable effect on the proportion of donor-type swarms. This was the result expected on the inferred order, since in each of these six crosses the fla mutation of the recipient lies between HI and that of the donor. Most possible crosses involving M6 fla-52 were made. The presence of a fla mutation in the donor did not consistently cause a great decrease of the proportion of donor-type swarms in any of these crosses; we are therefore unable to draw any firm inferences about the position of fla-52.

Recombination in the HI gene between serological mutants

All the crosses involving M6 (as donor) or M6 fla-52 (as recipient or as donor) yielded spreading growth on the plates which selected for fla+ transductants with the recombinant (wild type) antigen, and in most of these crosses swarms of this sort were abundant. Few crosses not involving M6 yielded swarms on such plates, but a very low proportion were observed in crosses of M5 with M10, of M10 with M11 and of M5 with M11. The serological identity of the phase-1 antigen of these transductants with the parental wild-type antigen was confirmed by the tests described under ‘Methods’; and all these transductants had the nutritional characters of their recipient parent. As no swarms were obtained on the control plates which tested for reversion of serological character, we conclude that the HI gene of these transductants is wild type, produced by recombination between the parental HI genes. Attempts to infer
Table 1. Crosses between serological and non-flagellate mutants

<table>
<thead>
<tr>
<th>Cross no.</th>
<th>Recipient</th>
<th>Donor</th>
<th>Highest dilution* with swarms with antigen i of type</th>
<th>Inference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M5 fla-50</td>
<td>M6 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M5 fla-50</td>
<td>M10 fla⁺</td>
<td>10⁻³ 10⁻² 10⁻¹ neg. 10⁻⁰</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>M5 fla-50</td>
<td>M12 fla⁺</td>
<td>10⁻³ 10⁻² 10⁻¹ neg. 10⁻⁰</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>M5 fla-50</td>
<td>M12 fla⁺</td>
<td>10⁻³ 10⁻² 10⁻¹ neg. sed. 10⁻⁰</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>M6 fla-52</td>
<td>M5 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M6 fla-52</td>
<td>M10 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M6 fla-52</td>
<td>M11 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M10 fla-57</td>
<td>M5 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M10 fla-57</td>
<td>M6 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
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<tr>
<td>10</td>
<td>M10 fla-57</td>
<td>M11 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M10 fla-57</td>
<td>M12 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M11 fla-55</td>
<td>M5 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
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</tr>
<tr>
<td>13</td>
<td>M11 fla-55</td>
<td>M6 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M11 fla-55</td>
<td>M10 fla⁺</td>
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</tr>
<tr>
<td>15</td>
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<td>M12 fla⁺</td>
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</tr>
<tr>
<td>16</td>
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<td>M5 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
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</tr>
<tr>
<td>17</td>
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<td>M10 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>M12 fla-58</td>
<td>M11 fla⁺</td>
<td>10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰</td>
<td></td>
</tr>
</tbody>
</table>

* Plates of semisolid medium containing appropriate sera (see Methods) were inoculated with standard-drop volumes of tenfold dilutions of transduction mixture, i.e. fla recipient and P22 grown on donor (phage: bacterium ratio 10:1). Entries record most dilute inocula giving swarms with indicated form of antigen i. (1 = undiluted mixture; sed. = mixture concentrated by centrifugation; neg. = no swarms of indicated type, even from inoculum of concentrate.)

† Inferences. A. fla of donor must lie between Ht and fla of recipient, because its presence causes hundredfold or greater reduction in proportion of fla⁺ transductants with donor Ht gene. B. fla of donor probably central, since it causes about a tenfold decrease in donor-type transductants. C. No indication that fla of donor is central, since it causes no consistent decrease in yield in donor-type transductants.
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The order of mutations within \( H_1 \) were unsuccessful, since the argument that recombinants with wild-type antigen probably arose by only two crossovers led to mutually contradictory map orders.

Recombination between a curly mutant and the serological mutants

The five crosses in which the donor carried an \( i \)-curly mutation and the recipient was a \( fla^+ \) derivative of a serological mutant all gave similar results (not included in Table I). Swarms were obtained from high dilutions (e.g. \( 10^{-5} \)) of the transduction mixture on plates not containing any anti-\( i \) serum. Swarms were also obtained on medium containing serum specific for the mutant \( i \) antigen of the recipient, but only from plates inoculated with the concentrated transduction mixture. Thus \( fla^+ \) transductants with the wild-type \( i \) antigenic character of the donor, but without its \( i \)-curly (and therefore phase-1 non-motile) character, formed only a very small proportion (\( 10^{-4} \) to \( 10^{-5} \)) of all \( fla^+ \) transductants in these crosses. This class presumably arose by crossing-over in \( H_1 \) between the mutation determining the curly character of the \( i \) flagellin of the donor and that determining the changed serological properties of the \( i \) flagellin of the recipient. Because of the rarity of recombinants with wild-type antigen and because a \( fla^+ \) mutant of the \( i \)-curly donor was not available no inferences could be drawn about the order within \( H_1 \) of the curly and altered-antigen mutations.

Control experiments confirmed the previously reported inseparability of the curly character from the determinant of phase-1 antigenic character in crosses between strains with unrelated phase-1 antigens. The \( S. \) typhimurium derivative \( 507 \), with \( b \) as its phase-1 antigen, was treated with phage \( P_22 \) lysates of several strains with antigen-\( i \), wild type or mutant in respect of serological character; numerous swarms with the donor phase-1 antigen were obtained on plates containing anti-\( b \) (and anti-\( 1,2 \)) serum. By contrast no \( i \) swarms were obtained when the donor carried the \( i \)-curly allele.

DISCUSSION

The mutations which altered the serological character of antigen-\( i \) (Joys & Stocker, 1963, 1966) presumably altered amino acids at serologically determinant sites in the polypeptide chain of \( i \) flagellin and we hoped to map the sites of the corresponding mutations within \( H_1 \), the structural gene for \( i \) flagellin. Motile (\( fla^+ \)) transductants with wild-type antigen-\( i \) were obtained in various crosses in which the recipient was \( fla^+ \) and each parent carried a mutant \( H_1 \) gene determining an altered form of antigen \( i \). We consider that the control experiments described prove that the wild-type antigen of these transductants arose by crossing-over within \( H_1 \), not by reversion. (We did not detect any recombinants of the reciprocal type, which would presumably have had the mutant serological specificities of both parents, but such recombinants would probably not have been detected by the method of serum selection used.) Transductants with wild-type antigen-\( i \) were abundant in most crosses involving \( M_6 \), either as donor or recipient, but rare or absent in all other crosses. We infer that the position of the \( M_6 \) mutation within \( H_1 \) is not very close to those of \( M_5, M_{10}, M_{11} \) and \( M_{12} \), and that these latter mutations are very close to each other. The clustering of most of the serological mutations within a small region of \( H_1 \) may be a consequence of the fact that all the mutants were isolated by selection with a single antiserum, which
would presumably tend to select mutants all of which had lost the same antigenic subfactor. Or it may be that only a small part of the polypeptide chain of flagellin is antigenically determinant.

In this system, in which various functionally efficient but serologically distinct forms of a single protein are available, it thus proved feasible to use the antigenic character of the protein for selection (and recognition) of intragenic recombinants. Attempts to infer the sequence of the mutations within $HI$ were, however, unsuccessful—because of the small numbers of wild-type $i$ recombinants obtained in crosses not involving $M6$ and because of the equivocal indications as to gene order provided by crosses of $M6 fla^+$ and $M6 fla-52$. In an earlier report (Joys & Stocker, 1963) we drew some conclusions about the order of sites within $HI$; we now consider those conclusions to be invalid since they relied on the premise that all quadruple cross-over classes would be very infrequent, a premise which no longer seems tenable, as discussed below.

In some ‘three-point’ crosses, the presence of a $fla$ mutation in the donor strain greatly decreased the proportion of $fla^+$ transductants with the donor-type antigen, whereas in other crosses there was no decrease. On the interpretation that the rare class requires four crossovers the data strongly indicate the order: $fla-50$—$fla-58$—$fla-57$—$fla-55$—$HI$. Complementation tests divide $fla$ mutants into groups (Joys & Stocker, 1965; Iino & Enomoto, 1966); $fla-55$ and $fla-57$ fall in complementation group $A$, $fla-58$ in group $D$ and $fla-59$ in group $B$. If each complementation group corresponds to a gene, the gene order indicated is $flaB$—$flaD$—$flaA$—$HI$, in agreement with that suggested by Iino & Enomoto (1966). In our earlier communication (Joys & Stocker, 1963) we placed $fla-55$ and $fla-52$ on the opposite side of $HI$ from $fla-50$, $fla-57$ and $fla-58$. The order now inferred is more plausible, in that it brings together $fla-55$ and $fla-57$, both of complementation group $A$, in which the earlier order were separated by $HI$. Our present data do not show the position of $fla-52$, but since this mutant also falls in complementation group $A$ its mutation is presumably in $flaA$, and so between $flaD-58$ and $HI$, a position indicated by later mapping experiments in which the $nmr$ gene served as external marker (U. Pearce & B. A. D. Stocker, unpublished). Our earlier inference that $fla-52$ and $fla-55$ were separated by $HI$ from the other $fla$ mutations was based on the premise of extreme rarity of quadruple cross-over recombinants. The more complete results now reported, especially those of crosses in which both parents are $fla$, show that this premise is incorrect, since reliance on it leads to inferred map orders which contradict each other.

The flagellins determined by ‘curly’ genes differ from normal flagellins by their tendency to form fibres of abnormally short wavelength on polymerization in vivo or in vitro. In one instance an alteration in the tryptic peptide map of the flagellin (Enomoto & Iino, 1966) proves that its ‘curly’ character results from mutation of its structural gene. In our crosses of an $i$-curly donor to various $fla$ recipients having $HI$ genes determining serologically altered forms of antigen-$i$ a very small fraction of the $fla^+$ transductants produced phase-I flagellin which was non-curly (as shown by the normal motility of the transductants when in phase 1) and of wild-type $i$ antigenic character (as shown by agglutination tests and by the failure of antiserum specific for the mutant $i$ antigen of the recipient to immobilize the transductants). The $HI$ genes of these transductants must have arisen by recombination, with a crossing-over between the mutation determining the curly character of the donor $HI$ gene
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and the serologically determinant mutation in the HI gene of the recipient. The very small proportion of fla+ transductants with the wild-type antigen may reflect merely the infrequency of the required crossover within HI—perhaps the curly mutation is close to the serologically determinant area. Alternatively it may be that in some or all the crosses the production of a fla+ transductant with wild-type antigen requires four crossovers, but we do not now consider that the data permit any reliable inference as to the order of the curly and serologically determinant mutations within HI. In the control experiment we did not obtain any transductants able to make normal i flagellin as a result of recombination of the i-curlly HI (donor) allele with a wild-type HI allele determining flagellin of antigenic type b. The amino acid composition of b flagellin differs substantially from that of i flagellin (McDonough, 1965) and the HI genes determining these flagellins must differ correspondingly in base sequence. Such non-homology would be expected to make recombination between these HI genes less frequent than when the donor and recipient HI genes determine almost identical flagellins.

REFERENCES


Note added in proof

Recently, S. Yamaguchi & T. Ino (J. gen. Microbiol. (1969), 55, 59) have demonstrated intragenic recombination between genes specifying various forms of the g. . antigen complex and have placed some antigenically important areas of the HI gene in a linear array.