SHORT PAPER

Location of a transduced *Salmonella* *H2* (phase-2 flagellin gene) region in *Escherichia coli* K-12

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ABSTRACT

The location of the *Salmonella* phase-2 flagellin gene and its associated regulatory elements, *vh2*⁺ *H2* *rh1*⁺ and *vh2* *H2* *rh1*⁺, transduced into *Escherichia coli* K-12 was determined by interrupted matings to be around 55 min on the linkage map. In P1 transduction with *vh2* *H2* *nalB*^r as donor, *tyrA*⁺ or *pheA*⁺ was cotransduced with *vh2* *H2* at frequencies of 3-6% but was not with *nalB*^r, suggesting that the integrated *H2* region interferes with cotransduction of *pheA* and *nalB* which are known as cotransducible, and also its likely location at the *nalB* side of *pheA*. On the other hand, the *vh2*⁺ *H2* region was not cotransduced with any markers tested and not transduced at all even when used as selective marker. This was discussed with relation to the constitution of the transducing fragment involving *vh2*⁺ *H2*. The delayed expression of *H2* in transductants selected for auxotrophic markers was also discussed.

1. INTRODUCTION

Most *Salmonella* species have two flagellin genes called *H1* and *H2* (Lederberg and Edwards 1953; Iino 1977) which are located at 41 and around 60 min, respectively, on the *Salmonella* linkage map (Sanderson and Hartman, 1978), and alternately expressed at a definite rate/bacterium/division (Stocker 1949), resulting in phase-1 or phase-2 flagella easily detected as antigen *i*, *e,n,x*, etc. The phenomenon is called phase variation (Andrews 1922; Lederberg and Iino 1956), one aspect of which is that the product of *rh1* (*H1* repressor, Fujita *et al.* 1973; Silverman *et al.* 1979) cotranscribed with *H2* represses *H1* when *H2* is expressed, resulting in phase-2, and the *H1* repression is released when *H2* becomes unexpressed by another mechanism (Silverman and Simon 1980), resulting in phase-1. On the other hand, *Escherichia coli* K-12 has one flagellin gene *hag48* (antigen *H48*) which is homologous to *Salmonella* *H1* (Mäkelä 1964) and always expressed. Transduction of *Salmonella* *H2* into *E. coli* K-12 by phage P1 (Enomoto and Stocker 1974) has revealed that most *H2* genes are transduced at the *hag* locus which has been previously replaced by *ah1* *H1*, without incorporation of linked *vh2* (variegater of *H2*, Iino 1961) and *rh1*,

and they behave as *H1* alleles in transduction back to *Salmonella* (Enomoto and Stocker 1975). The two *E. coli* transductants, however, were different from others; their *H2* was, when transduced back to *Salmonella*, recombined at the *H2* locus. They named EJ38 and EJ44 were found to receive *vh2⁺ H2-e, n, x rh1⁺* and *vh2 H2-e, n, x rh1⁺* from *Salmonella*, respectively, and show phase variation between 0 (flagellaless phase-1) and *e, n, x*; the *vh2* mutation which prevents phase variation in *Salmonella* becomes suppressed in a certain of *E. coli* (Enomoto unpublished data). The present report deals with the determination of *H2*-integration sites in the two *E. coli* transductants.

2. MATERIALS AND METHODS

Bacterial strains and phage: All the strains used (Table 1) are derivatives of *Escherichia coli* K-12 and the majority have been introduced *Salmonella H* region(s) of which *vh2⁺ H2 rh1⁺* and *vh2 H2 rh1* are of *S. abony* and *S. abortus-equi* origin, respectively. *ah1 H1* strains lack phase-1 flagella due to *ah1* (*H1* promoter) mutation (Iino 1977). *nalA^r* (*gyrA*) and *nalB^r* are resistant to nalidixic acid (Nal) of 40mg/l and 6mg/l, respectively (Hane and Wood 1969) and *nalB^r* mutants isolated were confirmed to be still sensitive to Nal (40mg/l). Hfr strains EJ262 and EJ275 were isolated by replica plating (Curtiss and Stallions 1969) from F⁺ derivatives of EJ44 and EJ38, respectively; the colonies

Table 1. *Bacterial strains used*

Strain	Relevant markers	Origin or source
EJ34	<i>ah1 H1-i</i>	W1485 (a) made <i>ah1 H1-i</i> by transduction from <i>Salmonella</i> (b)
HfrEJ262	<i>ah1 H1-i, vh2 H2-e, n, x rh1⁺, nalA^r</i>	EJ44 (b) made Hfr, then <i>nalA^r</i> by mutation (this study)
EJ271	<i>ah1 H1-i, lysA, rpsL</i>	EJ34 made <i>lysA rpsL</i> by the cross with F ⁺ JE3424 (c) (this study)
HfrEJ275	<i>ah1 H1-i, vh2⁺ H2-e, n, x rh1⁺, nalA^r</i>	EJ38 (b) made Hfr, then <i>nalA^r</i> by mutation (this study)
EJ281	<i>ah1 H1-i, vh2⁺ H2-e, n, x rh1⁺, nalB^r</i>	EJ38 made <i>nalB^r</i> by mutation (this study)
EJ282	<i>ah1 H1-i, vh2 H2-e, n, x rh1⁺, nalB^r</i>	EJ44 made <i>nalB^r</i> by mutation (this study)
EJ1351	<i>H1-i, tyrA2</i>	H680 (P. de Haan) transduced <i>H1-i</i> (this study)
JP190	<i>hag48, tyrA</i>	A. J. Pittard strain via W. H. Wood (d)
AT2092	<i>hag48, pheA</i>	same as the above
H888	<i>hag48, purG</i>	T. S. Matney strain via W. H. Wood (d)

References: a, Bachmann (1972); b, Enomoto and Stocker (1975); c, Abe and Tomizawa (1967); d, Wood and Egan (1972). Flagellin genes (*H1-i*, *H2-e, n, x* and *hag48*) used without a superscript + or - show wild-type alleles.

which gave rise to five or more *lysA*⁺ recombinants on the replicated selective plates on which the lawn of strain EJ271 was previously made, were isolated and further tested for marker entries and stability. Plvir was used for transduction throughout the experiments.

Media: E broth contained 1% polypeptone (Kyokuto), 0.5% meat extract (Kyokuto) and 0.5% NaCl. Synthetic medium used was M9 (Miller, 1972). Solid media contained 1.5% agar. Nutrient semisolid medium (NSS) contained 0.3% agar (Wako) in E broth. Anti-*i* and anti-*e, n, x* sera made against *Salmonella* monophasic strains were used for *H* transductants of *E. coli* without absorption by *Salmonella* somatic antigens. Anti-*H48* serum made against a K-12 wild strain W3110 was used after absorption by its Fla mutant.

Conjugation and transduction: Mating procedures were as described by Hayes (1968). The interruption was performed by blending 10-fold dilutions of the mating mixture for 30 sec with Micronizer (Nihonseiki). Samples at each interruption time were diluted 100-fold into buffered saline (0.85% NaCl in one-tenth concentration of M9 salts) and stocked in ice. To detect *H2* recombinants, samples (0.1–0.5 ml) were quickly mixed in plates with cooled but not solidified NSS containing anti-*i* serum and streptomycin (Sm, 200 mg/l) and after incubation at 37°C swarms (motile recombinants) were counted under a binocular microscope while they are still small. For *nalA*⁺ detection samples were layered with 2 ml water agar (0.5%) on minimal agar plates supplemented with lysin (20 mg/l) and Sm, incubated for 2 hr at 37°C and then 2 ml water agar containing Nal (500 mg/l) was layered. For transduction Plvir grown on *H2*^{on} donor bacteria was mixed with a recipient culture containing 5 mM CaCl₂ at moi less than 0.1. The mixture was centrifuged after 30 min adsorption, resuspended in one-fifth volume of buffered saline and plated on selective media containing 10 mM sodium citrate. For *H2* transduction the centrifugal pellet was resuspended in one-fifth volume of E broth containing 10 mM sodium citrate and inoculated on NSS containing anti-*i* serum the way to draw lines. To test for *nalB*⁺ inheritance, transductants for each auxotrophic marker were inoculated as patches onto the same selective plate and replica plated on nutrient agar containing Nal (6 mg/l).

3. RESULTS AND DISCUSSION

Mapping of H2 in *E. coli* by interrupted matings: To determine the *H2* sites in the two *E. coli* transductants EJ38 and EJ44, Hfr's were isolated from these strains. HfrEJ262 isolated from EJ44 had the origin of transfer at around 65 min and transferred the chromosome counterclockwise. HfrEJ275 from EJ38 showed the clockwise transfer from the origin at around 35 min.

Fig. 1. Interrupted mating of A, HfrEJ262 × EJ271 and B, HfrEJ275 × EJ271. For the method of mating interruption and the detection of *H2* and *nalA*^r recombinants see Materials and Methods.

These Hfr's were made *nalA*^r and used for interrupted matings with an F⁻ strain EJ271 (*ah1 H1, lysA, rpsL*). The cross HfrEJ262 × EJ271 indicated that *lysA*⁺ entered at 4 min, *H2-e, n, x* at 12 min and *nalA*^r at 23.5 min, and the cross with HfrEJ275 indicated the reverse entries of *nalA*^r (16.5), *H2* (28) and *lysA*⁺ (37 min) as expected (Fig. 1). The time interval between *lysA* and *nalA* entries was 19.5 min with HfrEJ262 and 20.5 min with HfrEJ275, while on the *E. coli* linkage map it is estimated to be 13 min (Bachmann and Low 1980), showing that the chromosome transfer in this mating system is about 1.5 times as slow as the standard. The location of *H2* was calculated by proportional allotment after the distances between *lysA* and *nalA* were standardized; *H2* was located at about 55 min on the standard linkage map in both Hfr strains.

Cotransduction of H2 and auxotrophic markers: To ascertain the inferred *H2* location, cotransduction of *H2* with neighboring genes was attempted.

Table 2. Cotransduction of *H2* and neighboring genes

Recipient	Selected marker	EJ281 donor		EJ282 donor	
		No. of transductants with unselected		No. of transductants with unselected	
		<i>H2</i> (%)	<i>nalB</i> ^r (%)	<i>H2</i> (%)	<i>nalB</i> ^r (%)
<i>H1-i tyrA2</i>	<i>tyrA</i> ⁺	0/416 (<0.2)	NT	12/212 (5.7)	NT
<i>hag48 tyrA</i>	<i>tyrA</i> ⁺	0/456 (<0.2)	0/240 (<0.4)	15/456 (3.3)	0/240* (<0.4)
<i>hag48 pheA</i>	<i>pheA</i> ⁺	0/380 (<0.3)	0/180 (<0.6)	19/560 (3.4)	0/180* (<0.4)
<i>hag48 purG</i>	<i>purG</i> ⁺	0/353 (<0.3)	NT	0/421 (<0.2)	NT

To test for *H2* inheritance, transductants selected for each auxotrophic marker were stabbed in NSS containing anti-flagella serum for the recipient, and production of swarms was examined after overnight incubation for the *H1-i* recipient and after 3 days for the *hag48* recipients. **H2* transductants are included. NT, not tested.

purG (53.5), *pheA* (56), *tyrA* (56) and *nalB* (57 min) were chosen to be tested. EJ281 (*vh2*⁺ *H2* *rh1*⁺ *nalB*^r) and EJ282 (*vh2* *H2* *rh1*⁺ *nalB*^r), the *nalB*^r derivatives of EJ38 and EJ44, respectively, were used as donor. The wild-type transductants for each auxotrophic marker were tested for the donor *H2-e, n, x* and *nalB*^r markers (Table 2). The EJ282 donor indicated cotransduction of *tyrA*⁺ and *H2*, and of *pheA* and *H2* at frequencies of 3.6% and 3.4%, respectively, whereas the EJ281 donor did not. With both donors cotransduction between *tyrA* or *pheA* and *nalB* was not detected, though *pheA* and *nalB* have been reported to be cotransducible at a frequency of about 5% (Woods and Egan 1972). The transduction frequency for the four kind of auxotrophic markers was $2.5-8.0 \times 10^{-7}$ per pfu and not significantly different for each marker between the two donors. These tests did not clarify the *H2* integration site in *E. coli* since the three-point transduction involving *tyrA*, *H2* and *nalB* was not practicable. When *H2* is located at around 55 min between *purG* and *pheA* as inferred from the interrupted matings, *purG* and *H2* would be expected to show cotransduction at a low frequency, because the distance between *purG* and *pheA* is about 2.5 min and the 3% cotransduction frequency between *pheA* and *H2* corresponds to the distance of about 1.4 min (Wu 1966) unless heterogeneity between the recipient chromosome and the donor fragment is taken into account. Cotransduction of *purG* and *H2*, however, was less than 0.3%. On the other hand, when *H2* is located at the *nalB* side of *pheA*, the inserted *H2* region would hinder cotransduction of *pheA* (or *tyrA*) and *nalB* which ought to be cotransduced. In this case *H2* would be situated between *pheA* and *nalB* since all the *pheA*⁺ *H2* and *tyrA*⁺ *H2* transductants were still *nalB*^r.

H2 of the EJ281 donor was not cotransduced with any markers tested, though it was located at 55 min, the site identical with *H2* of EJ282. A plausi-

ble explanation is that in EJ281, the original *H2* transductant, a larger *Salmonella* transducing fragment is integrated into the *E. coli* genome, and in next transduction with EJ281 as donor the greater part of the transducing fragment carrying *H2* again becomes *Salmonella* origin, causing the reduced frequency of recombination as observed in initial transduction from *Salmonella* to *E. coli* (Enomoto and Stocker 1975). To test this assumption the *H2* region was directly transduced from both donors to the *ah1 H1* strain, EJ34. The EJ282 lysate evoked *H2* transductants at about 3×10^{-9} per pfu, whereas the EJ281 lysate did not evoke any *H2* transductant in repeated experiments, the frequency being estimated at less than 2.6×10^{-10} per pfu, though the same lysate evoked as many transductants for the auxotrophic markers as EJ282 did. The result seems to favor the proposed assumption.

In the course of the experiments a noticeable phenomenon was revealed. The transductants obtained from the *H1-i tyrA* recipient produced swarms (*e, n, x^{on}*) in NSS containing anti-*i* serum after overnight incubation when they received *H2-e, n, x* and showed phase variation between *i* and *e, n, x*; while those from the *hag48* recipients produced swarms in NSS containing anti-*H48* serum only after incubation for 2-3 days and these swarms (*e, n, x^{on}*), when restabbed in NSS containing anti-*e, n, x* serum, did not produce swarms (*H48^{on}*) any more and instead showed phase variation between 0 and *e, n, x*. It will be aside from the purpose of this report to describe the detailed analysis of the phenomenon; which, however, would be simply explained by the following assumption: *Salmonella rh1* does not repress *E. coli hag48* and accordingly, the *H2* transductant is able to express the flagellar phase of either *H48^{on}* *e, n, x^{on}* or *H48^{on}* *e, n, x^{off}*, motility of both phases being hindered by anti-*H48* serum, and motile colonies which overcome the inhibition by serum ought to have some mutations in *hag48*, indicating variation between 0 and *e, n, x*.

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