Chromosomal Location of Host Specificity in *Salmonella typhimurium*

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SUMMARY

The chromosomal location of the genes for host specificity in *Salmonella typhimurium* has been investigated by F-mediated conjugation using host specificity mutants isolated previously. It was found that the sites of mutations leading to two distinct phenotypes, $r_{LT}^-m_{LT}^+$ and $r_{LT}^-m_{LT}^-$, are closely linked to each other and are located near the marker *proC*.

INTRODUCTION

We have recently isolated a series of mutants of *Salmonella typhimurium* which are defective in a system of host-controlled restriction and modification that controls the acceptance of foreign phage P22 DNA and bacterial DNA (Colson, Colson & van Pel, 1969). These mutants closely resemble those isolated in *Escherichia coli* K and B in which recombination and complementation studies have shown that the functions of restriction and modification are governed by a cluster of at least three genes situated at about one time unit to the left of the marker *thr* (Colson, Glover, Symonds & Stacey, 1965; Wood, 1966; Glover, 1968; Arber & Linn, 1969; Boyer & Roulland-Dussoix, 1969; Glover & Colson, 1969). In view of the extensive homology observed between the genetic maps of *E. coli* and *S. typhimurium* (Sanderson & Demerec, 1965), one may consider *a priori* that this locus is a good candidate for the chromosomal location of the host specificity genes in *S. typhimurium*. On the other hand, the loss of the genetic determinants for host specificity in *S. typhimurium* was first observed in *lac*⁺ 'hybrids' obtained from conjugation experiments between a Hfr *E. coli* and *S. typhimurium* LT7 *mut* (harbouring a mutator gene). Therefore, it was thought that these genetic determinants were lost by exchange of genetic material with *E. coli* in the *pro-lac* region of the map (Zinder, 1960). However, recent experiments show that crosses between *E. coli* Hfr and *S. typhimurium* tend to select host specificity deficient mutants in the recipient strain (Colson & Colson, 1967; Okada, Watanabe & Miyake, 1968).

The experiments presented in this paper define the map position of the genes for host specificity in *Salmonella typhimurium* by means of bacterial conjugation with host specificity mutants isolated in F⁻ and Hfr strains.

METHODS

Bacteria. The bacterial strains used in this study are listed in Table 1, and the map position of the relevant markers is shown in Fig. 1.

Media. The media have been described previously (Colson, Colson & van Pel, 1969).

Scoring of host specificity phenotype. Restriction and modification were scored with a *c2* mutant of phage P 22. The restriction test was performed by inoculating small broth cultures of the strains in soft agar overlays on plates and then adding drops of

Fig. 1. *Partial linkage map of Salmonella typhimurium.* The point of origin and the direction of transfer by HfrK 1 and HfrK 4 are indicated by arrows. The details of the *pro* region are from Itakawa & Demerec, 1968. *proA* 46 and *proC* 90 are point mutations, *proC* 110 is a deletion covering most of *proC*, *proAB* 47 is a deletion extending across *proA*, *proB*, and the attachment site of phage P 22 (*attP* 22). The orientation of the whole *pro* region with respect to outside markers is not certain (Smith, 1968).

Table 1. *Bacterial strains*

Symbol	Genotype*	Host specificity mutations†	Source
SA 534 HfrK 4	<i>serA</i> 13	<i>hsr-51</i> <i>hss-53</i> <i>hss-91</i>	K. E. Sanderson
SA 464 HfrK 1	<i>serA</i> 13	—	K. E. Sanderson
SA 195	<i>proAB</i> 47 <i>purE</i> 66	<i>hsr-113</i> <i>hss-8</i> <i>hss-617</i>	K. E. Sanderson
SB 106	<i>ile-405 rha-461 leu-1003</i> <i>proA-46 purC7 str-r</i>	<i>hsr-37</i>	P. E. Hartman
—	<i>proC</i> 90	<i>hsr-185</i> <i>hss-4</i> <i>hss-16</i> <i>hss-64</i> <i>hss-800</i>	H. O. Smith
—	<i>proC</i> 110	<i>hss-6</i> <i>hss-20</i>	H. O. Smith

* The map position of the markers used in this study are indicated in Fig. 1.

† The gene symbols used for host specificity are in agreement with the terminology of Arber & Linn, 1969. Mutants which have lost the capacity to restrict foreign DNA while retaining the capacity to modify it specifically (phenotype $r_{LT}^+m_{LT}^+$) are designated *hsr*. Mutants which have lost both the capacities to restrict and to modify DNA as the result of a single mutation (phenotype $r_{LT}^-m_{LT}^-$) are designated *hss*. The isolation and the phenotypic characterization of these mutants have been described previously (Colson, Colson & van Pel, 1969).

serially diluted suspensions of P 22 c 2.LT (having the LT modification) and P 22 c 2.0 (lacking the LT modification). After incubation, strains able to restrict (r_{LT}^+) show 10^4 less plaques of P 22 c 2.0 than of P 22 c 2.LT, while strains unable to restrict (r_{LT}^-) accept both phages with the same efficiency. For scoring large numbers of recombinants a quicker method was utilized. Single colonies of the restreaked recombinants were resuspended in drops of buffer and cross-streaked on nutrient agar against streaks of suspensions containing 10^5 plaque-forming units/ml. of P 22 c 2.LT and P 22 c 2.0. After incubation, r_{LT}^- strains show confluent lysis at both phage streaks while r_{LT}^+ strains are lysed only by P 22 c 2.LT. The modification test was performed by adding

Table 2. *Linkage between ile, leu, pro, and hsr in the cross:*
HfrK 1 × SB 106 ile leu proA hsr-37 str-r

Exponentially growing cultures of the donor and recipient strains were mixed in equal volumes, centrifuged and kept at 37° for 60 min. without resuspending the pellets. The mixture was resuspended, diluted and plated on selective minimal media containing 100 µg./ml. of streptomycin. The recombinants were purified by single colony isolation on the same media and then tested individually for unselected markers.

Selected marker	Unselected markers (%)				No. of colonies tested
	<i>ile</i> ⁺	<i>leu</i> ⁺	<i>pro</i> ⁺	<i>hsr</i> ⁺	
<i>ile</i> ⁺	—	6	4	3	97
<i>leu</i> ⁺	41	—	35	32	99
<i>pro</i> ⁺	41	49	—	63	99

Table 3. *Inheritance of hsr⁺ from HfrK 1 among various classes of recombinants in the leu-pro-purE region*

Same procedure as in Table 2, except that the purified recombinants were first classified with respect to the inheritance of unselected nutritional markers before being tested for their restriction phenotype.

Recipient strains	Classes of recombinants	<i>hsr</i> ⁺ (%)	No. of colonies tested
SB 106 <i>hsr-37</i> <i>ile leu proA str-r</i>	<i>leu</i> ⁺ <i>pro</i> ⁺	56	83
	<i>leu pro</i> ⁺	66	50
	<i>leu</i> ⁺ <i>pro</i>	23	64
SA 195 <i>hsr-113</i> <i>proAB purE str-r</i>	<i>pro</i> ⁺ <i>pur</i> ⁺	97	99
	<i>pro</i> ⁺ <i>pur</i>	88	75
	<i>pro pur</i> ⁺	< 2	46

drops of serially diluted suspensions of P 22 c 2 grown on the strain to be tested to plates seeded with soft agar overlays of known r_{LT}^+ and r_{LT}^- strains. After incubation, an equal number of plaques is observed on both indicators if the phage carried the modification, indicating that the test strain was m_{LT}^+ . On the contrary, the phage from a m_{LT}^- strain form 10^4 less plaques on the r_{LT}^+ indicator than on the r_{LT}^- . For the scoring of a large number of recombinants, this test was simplified by using drops of buffer instead of tubes and calibrated loops instead of pipettes for diluting and spotting the phage suspensions.

RESULTS

Mapping the hsr mutation. About 100 *ile*⁺, *leu*⁺ and *proA*⁺ recombinants were selected independently from a cross between HfrK 1 and the multiple auxotroph SB 106 recipient strain carrying a presumed *hsr* mutation (phenotype *r_{LT}m_{LT}⁺*). These recombinants were analysed for the inheritance of unselected donor markers including the wild-type host specificity marker. The results show that *hsr*⁺ is inherited together with *pro*⁺ (63 %) while its linkage to *leu*⁺ (32 %) and *ile*⁺ (3 %) is almost the same as that of *pro*⁺ to those markers (Table 2). The close linkage of the *hsr* gene to the *pro* region was confirmed by the results of two other crosses performed using HfrK 1. In the first cross with SB 106 *hsr*, *leu*⁺ and *proA*⁺ recombinants were selected while *proAB*⁺ and *purE*⁺ recombinants were selected in the second cross to SA 195 *hsr*. From the first cross *leu*⁺ *pro*⁺, *leu pro*⁺ and *leu*⁺ *pro* recombinants were obtained and the inheritance of *hsr*⁺ was scored among each class of recombinants. Similarly in the second cross *pro*⁺ *pur*⁺, *pro*⁺ *pur* and *pro pur*⁺ recombinants were obtained and the inheritance of *hsr*⁺ was scored among each class. In both crosses whenever *pro*⁺ was inherited by the recombinant class the inheritance of *hsr*⁺ was high. When selection was made against the inheritance of *pro*⁺ the inheritance of *hsr*⁺ was low (Table 3).

In an attempt to locate the site of *hsr* mutations more precisely with respect to the *pro* cistrons, two interrupted mating experiments were conducted using HfrK 4 and selecting *proA*⁺ recombinants in one cross while selecting *proC*⁺ recombinants in the other. Both recipient strains were *hsr* mutants and the inheritance of *hsr*⁺ was scored among *pro*⁺ recombinants selected at 2 min. intervals. The percentage of *hsr*⁺ among *proA*⁺ increased with the time allowed for transfer, rising to 88 % at a blending time of 36 min., while the percentage of *hsr*⁺ among *proC*⁺ was at the maximum level even at the earliest time of blending (Table 4). This means that when *hsr*⁺ is transferred by

Table 4. *Inheritance of pro⁺ and hsr⁺ in interrupted mating experiments with HfrK 4*

1 ml. samples of the exponentially growing recipient strains were mixed with equal volumes of 1/10 dilutions of exponentially growing cultures of the donor and the cells were immediately collected by filtration on a millipore filter. The filters were kept for 10 min. on the surface of a soft nutrient agar plate at 37°. The filters were then transferred to large flasks containing 20 ml. of prewarmed broth and gently agitated in a shaking water bath at 37°. At intervals, a 1 ml. sample was diluted into 9 ml. of buffer, shaken vigorously for 30 sec. Samples of 0.1 or 0.5 ml. of the blended mixtures were added to 3 ml. of melted soft agar containing 100 µg./ml. of streptomycin and immediately poured over the surface of the selective media containing the same concentration of streptomycin. About 50 *pro*⁺ recombinants from each sample were purified and scored for their host specificity phenotype.

Time of blending (min.)	Recipient strains			
	SB 106 <i>proA</i> 46 <i>hsr</i> -37		<i>proC</i> 90 <i>hsr</i> -185	
	No. of recombinants/ml.	<i>hsr</i> ⁺ (%)	No. of recombinants/ml.	<i>hsr</i> ⁺ (%)
24	1.3 × 10 ³	56	5.8 × 10 ³	90
26	4.3 × 10 ³	56	8.8 × 10 ³	> 98
28	8.0 × 10 ³	60	2.1 × 10 ⁴	90
30	1.5 × 10 ⁴	81	3.4 × 10 ⁴	> 98
32	2.0 × 10 ⁴	62	6.3 × 10 ⁴	98
34	6.5 × 10 ⁴	78	1.1 × 10 ⁵	92
36	6.4 × 10 ⁴	88	1.9 × 10 ⁵	98

HfrK 4 it enters the recipient cell after *proA*⁺ and either before *proC*⁺ or so closely after it that it cannot be separated from it by blending. Thus *hsr* maps either between *proA* and *proC* or just to the right of *proC*. In addition, this experiment suggests that the orientation of the *pro* region is: *leu-proB proA proC-purE*, since it is the only orientation compatible with the fact that *hsr* is closer to *proC* than to *proA*, while distal to *proA* when transferred by an Hfr injecting its chromosome in the order *leu-pro-purE*.

Linkage between hsr and hss mutations. The experiments presented in the previous section involved *hsr* mutants only. No conclusion can therefore be drawn from them about the chromosomal location of the presumed *hss* mutation leading to the $r_{LT}^-m_{LT}^-$ phenotype, although by analogy, one might expect to observe clustering of the genes

Table 5. Host specificity among *pro*⁺ recombinants in crosses between *hss* and *hsr* mutants

The *pro*⁺ recombinants were obtained by the method described in Table 2 and scored for their restriction and modification phenotype.

Expt. no.	Donors	Recipients	No. of recombinants with genotype			Donor type (%)
			<i>hss</i> ⁺ <i>hsr</i> ⁺	<i>hsr</i>	<i>hss</i>	
1	HfrK 4 <i>hss-53</i>	<i>proC</i> 90 <i>hsr-185</i>	2	5	193	96
		<i>proAB</i> 47 <i>hsr-113</i>	0	8	192	96
2	HfrK 4 <i>hss-91</i>	<i>proC</i> 90 <i>hsr-185</i>	18	49	344	83
		<i>proAB</i> 47 <i>hsr-113</i>	1	14	83	85
3	HfrK 4 <i>hsr-51</i>	<i>proC</i> 110 <i>hss-6</i>	0	35	13	73
		<i>proC</i> 90 <i>hss-4</i>	0	48	2	96
		<i>proC</i> 90 <i>hss-16</i>	0	24	12	66
		<i>proC</i> 90 <i>hss-64</i>	0	38	10	79

for host specificity in *Salmonella typhimurium* similar to that found in *Escherichia coli*. To locate the *hss* mutation *proC*⁺ and *proAB*⁺ recombinants were selected in crosses between *hss* mutants of HfrK 4 and *hsr* mutants of SA 195 and *proC* 90. In each cross more than 80% of the *pro*⁺ recombinants had inherited the donor $r_{LT}^-m_{LT}^-$ phenotype (Expts. 1 and 2, Table 5). Similarly, in crosses between an *hsr* donor and *hss* recipients most *pro*⁺ recombinants expressed the $r_{LT}^-m_{LT}^+$ phenotype of the donor (Expt. 3, Table 5). This shows that both *hsr* and *hss* mutations are closely linked to *pro*. Moreover, the small number of recombinants expressing wild-type host specificity (3% or less) indicates that the sites of the *hsr* and *hss* mutations are closely linked since recombination between them to restore wild-type host specificity is a rare event (Table 5).

Crosses were also performed between an Hfr expressing wild-type host specificity and *hss pro* recipients. Surprisingly no inheritance of wild-type host specificity among *pro*⁺ recombinants was observed for most of them (Table 6). Some *hss*⁺ recombinants were found in only two cases (*hss-6* and *hss-4*) and at frequencies of 11 and 78% respectively. These results may reflect the existence of several mutational sites leading to the $r_{LT}^-m_{LT}^-$ phenotype scattered along the chromosome and in most cases not transferred at a measurable frequency from HfrK4. Nevertheless, the following observations render this hypothesis unlikely. First, on repetition of the crosses in which a measurable linkage between *hss* and *pro* had been observed, the values

obtained proved to be very variable (less than 2% instead of 11% for *hss-6* and 8% instead of 78% for *hss-4*, see Table 6). This indicates that these linkage values cannot be regarded as a true reflexion of map distances. Second, four *hss* recipient strains in which the linkage between *hss* and *pro* was variable or absent were crossed with a *hsr* Hfr. All of them inherited the donor host specificity phenotype at a high frequency among *pro*⁺ recombinants indicating that the *hss* mutation they carry is closely linked to the *hsr* mutation (see Table 5).

Table 6. *Inheritance of hss⁺ from HfrK 4 among pro⁺ recombinants*

Same procedure as in Table 2; for each cross the results of two independent experiments are listed.

Recipient strains	No. of <i>hss</i> ⁺	No. of colonies tested
<i>proC 110 hss-6</i>	12	104
	0	50
<i>proC 110 hss-20</i>	0	24
<i>proC 90 hss-4</i>	78	100
	4	50
<i>proC 90 hss-16</i>	0	124
	0	50
<i>proC 90 hss-64</i>	0	24
	0	50
<i>proC 90 hss-800</i>	0	104
	0	50
<i>proAB 47 hss-617</i>	0	24
	0	50
<i>proAB 47 hss-8</i>	0	32

DISCUSSION

Mapping experiments using F-mediated conjugation in *Salmonella typhimurium* have shown that the sites of the *hsr* and *hss* mutations of the LT host specificity system are very close to each other and map in the vicinity of the marker *proC*. It must be pointed out that the use of the gene symbols *hss* and *hsr* was dictated only by the close phenotypic resemblance of the mutants in the LT system to those of *Escherichia coli* K and B, and that the experiments presented here give no information about the number and the functions of the genes. However, our experiments enhance the similarity between the *S. typhimurium* LT and the *E. coli* systems in showing that mutations leading to distinct phenotypes are clustered. Crosses between mutants have yielded recombinants between *hss* and *hsr* at a higher frequency than between similar mutants in *E. coli* (Lederberg, 1966; Glover & Colson, 1969). It is thus possible that the genes for the LT host specificity are less clustered than in *E. coli* or, perhaps more likely, it reflects the better resolving power of recombination analysis by conjugation in *S. typhimurium* which results from the lower rate of DNA transfer by *S. typhimurium* donors (Sanderson & Demerec, 1965).

On the other hand, our experiments show some differences between *Salmonella typhimurium* LT host specificity and host specificity in *Escherichia coli*. The almost complete absence of recombinants expressing the donor type host specificity in crosses between wild-type donors and *hss* recipients was not observed in similar crosses in

E. coli K and B. The explanation of this phenomenon remains obscure although the fact that it occurred *only* in crosses between wild-type donors and *hss* recipients suggests that this phenomenon may be related to the special situation in zygotes in which the resident DNA is not modified and which have freshly inherited a set of genes governing the ability to degrade this DNA.

The difference in map position between the LT host specificity genes and those of *Escherichia coli* suggests that this host specificity system may have a different origin. Moreover, experiments to be published have revealed that *Salmonella typhimurium* harbours another host specificity in addition to the LT system, the genetic determinants of which behave as allelic to those of the *E. coli* host specificities.

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