A New *Salmonella typhimurium* DNA Host Specificity

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SUMMARY

The genetic properties of a new (*hspS*) host specificity of *Salmonella typhimurium* were investigated using bacteriophage L. Phage L is a better substrate for s-specific restriction than phage P22. Mutants deficient in s-restriction only were found at the same frequency as mutants deficient in both restriction and modification. Crosses between *S. typhimurium* Hfr and *S. typhimurium* F⁻ or between *Escherichia coli* and *S. typhimurium* showed that the s system has the same chromosomal location as the κ system of *E. coli*. The s system was introduced into *E. coli* and found to be effective on phage λ .

INTRODUCTION

Escherichia coli strains K12 and B display different host-specific restrictions and host-specific modifications of DNA which, nevertheless, depend on very similar functions. These functions are governed by a gene cluster which has the same chromosomal location in both strains (Boyer, 1964; Wood, 1966; Glover & Colson, 1969). Studies with merodiploids have demonstrated that some of these functions are not strain specific but can complement mutants in the corresponding gene of the other strain (Boyer & Roulland-Dussoix, 1969; Glover, 1970). A third type of host specificity system, the A system, is present in *E. coli* 15T⁻ and was recently found to have the same chromosomal location as those of K12 and B (Arber & Wauters-Willems, 1970). One may reasonably expect that examination of more strains will reveal the existence of other related systems.

This paper will demonstrate such a system in strains LT7 and LT2 of *Salmonella typhimurium*. We report in a separate paper (Colson & Colson, in preparation) that recombinants between *Escherichia coli*-Hfr and *S. typhimurium* which had inherited either the *E. coli* κ or B system produced, after infection, a phage P22 which plated slightly less efficiently on *S. typhimurium* than on the *E. coli*/*S. typhimurium* recombinant on which it had been grown. The observation that phage L, closely related to P22 (Bezdek & Amati, 1967) underwent a higher (100-fold) reduction in efficiency of plating (e.o.p.) under the same conditions led us to postulate the existence of a host specificity system in *S. typhimurium* in addition to the previously studied LT system (Colson, Colson & Van Pel, 1969; Colson, Colson & Van Pel, 1970). By using phage L as the test phage for this new system, which we have called 's', it was possible to isolate mutants defective in s host specificity and to show that the genes (*hspS*) for the s system have the same chromosomal location as those for the κ system in *E. coli*. It was also found that *hspS* can be transferred to *E. coli* where it restricts and modifies phage λ .

METHODS

Bacteria. The bacterial strains used in this study were derivatives of *Escherichia coli* K12 and B and *Salmonella typhimurium* LT2 and LT7, and recombinants between *E. coli* and *S. typhimurium* LT7 (see Table 1).

Bacteriophages. The *Salmonella typhimurium* phages used were P22 c2, a clear plaque mutant of P22 and Lc, a spontaneous clear plaque mutant of phage L (Bezdek & Amati, 1967) received from P. Amati. The male-specific phage MS2 was used to test for the presence of the F factor in bacterial strains. The notations recommended by Arber & Linn (1969) for host specificity types and the modification carried by phages were followed throughout.

Media. The media used for the growth of *Salmonella typhimurium* strains and the plating of *S. typhimurium* phages have been described (Colson *et al.* 1969). The *Escherichia coli* strains were grown in L-broth, containing per litre: Difco Bacto Yeast Extract, 10 g.; Difco Bacto Tryptone, 10 g.; NaCl, 5 g. L-Broth solidified with 1% Difco Bacto Agar was used as bottom agar for the plating of phage λ .

Bacterial crosses. Crosses between *Salmonella typhimurium* Hfr and F⁻ strains were performed by mixing equal volumes of exponentially growing cultures, centrifuging the cells and keeping the centrifuge tubes for 1 h. at 37° before resuspending the pellets. The recombinants were selected on minimal medium and purified by single colony isolation on the same medium before analysis.

Scoring of host specificity phenotypes. The restriction and modification phenotypes were determined as described previously (Colson *et al.* 1970). The e.o.p. of phage λ was measured

Table 1. *Bacterial strains*

Strain number	Genotype*†	Restriction and modification phenotype‡	Origin
1200	<i>thr leu lac</i>	$r_K^+ m_K^+$	<i>Escherichia coli</i> K12 strain c600
1209	<i>thr leu lac hspK</i>	$r_K^- m_K^-$	From 1200 (Colson <i>et al.</i> 1965)
2000		$r_B^+ m_B^+$	<i>E. coli</i> B strain bc251 (Arber & Dussoix, 1962)
2007	<i>leu, ilv serB lac hspB</i>	$r_B^- m_B^-$	From 2000 (Glover & Colson, 1969)
4109		$r_{LT}^+ m_{LT}^+$	<i>Salmonella typhimurium</i> LT7
4233	<i>proC90</i>	$r_{LT}^+ m_{LT}^+$	From K. Sanderson
4253	<i>proC90 hspLT</i>	$r_{LT}^- m_{LT}^-$	From 4233 (Colson <i>et al.</i> 1969)
4256	<i>proC90 hspLT</i>	$r_{LT}^- m_{LT}^+$	From 4233 (Colson <i>et al.</i> 1969)
4441	<i>proC90 thr leu hspLT hspS</i>	$r_{LT}^- m_{LT}^+ r_S^- m_S^+$	From 4256
4247	<i>metA metE trpB</i>	$r_{LT}^+ m_{LT}^+$	<i>S. typhimurium</i> LT2 strain SL1027 from B. Stocker
4274	<i>metA metE trpB hspLT</i>	$r_{LT}^- m_{LT}^+$	From 4247 (Colson <i>et al.</i> 1969)
4214	Hfr K4 <i>serA13</i>	$r_{LT}^+ m_{LT}^+$	From K. Sanderson
4304	<i>(mut⁺leu⁺ara hspK⁺) hspLT</i>	$r_{LT}^- m_{LT}^- r_K^+ m_K^+$	<i>E. coli</i> × <i>S. typhimurium</i> recombinants (Colson & Colson, in preparation)
4321	<i>(mut⁺leu⁺ara hspK⁺) hspLT</i>	$r_{LT}^- m_{LT}^- r_K^- m_K^+$	
4328	<i>(mut⁺leu⁺ara⁺pyrA hspK⁺) hspLT</i>	$r_{LT}^- m_{LT}^- r_K^- m_K^+$	

* The genetic symbols are those of Taylor & Trotter (1967).

† Bracketed markers are those inherited from *E. coli*.

‡ Notation of host specificity phenotype is as Arber & Linn (1969).

with indicator bacteria grown in L-broth plus 0.2% maltose and starved for 1 h. at 37° in 0.01 M-MgSO₄ prior to phage infection. The phages were preadsorbed for 15 min. before plating.

RESULTS

Mutants of the s system. Restriction-deficient mutants of the s system were isolated by the same methods as those used previously for the isolation of mutants in the LT system (Colson *et al.* 1969). About 1×10^3 cells from nitrosoguanidine treated cultures were plated together with 1×10^5 particles of Lc. K (grown on a recombinant possessing the κ system of *Escherichia coli*). The plates were examined for infected colonies, recognizable by their nibbled appearance, which were restreaked and tested for the capacity to exercise s restriction and modification. About half of them were found to lack either the s restriction only or both s restriction and modification. More mutants were then isolated by using phage Lc. O (grown on a mutant lacking the s modification). Some mutants were found also among clones successfully infected with F-lac⁺ after mating with *E. coli* but this method was less effective than it was in selecting mutants in the LT system, probably because s restriction reduces the frequency of F-lac⁺ infection much less than does LT restriction.

Among 21 mutants isolated independently (Table 2), 11 had lost both s restriction and modification ($r_s^-m_s^-$) while 10 had lost only the s restriction ($r_s^-m_s^+$). Some $r_s^-m_s^-$ mutants were obtained in strains which already lacked the LT restriction and modification thus making it possible to demonstrate the independent action of the s and LT restrictions on phages without the use of recombinants between *Escherichia coli* and *Salmonella typhimurium*. Table 3 shows the results of this analysis for phages P22 and L. When these phages lack s modification they exhibit the same reduction in e.o.p. on a $r_s^+m_s^+ r_{LT}^-m_{LT}^-$ strain as phage with the κ or κ modification. Thus this reduction of e.o.p. results from the absence of the s modification and not from the presence of an *E. coli* modification. It appears also that the s and LT restrictions are additive: the restriction coefficient of phage L. O is higher on a strain exercising both restrictions than on strains exercising only s or LT restriction. Nevertheless, the restriction coefficient of P22.0 by a strain exercising both s and LT restrictions is not lower than that of L. O in spite of the fact that P22 DNA is a poor substrate for the s restriction. This seems to be caused by a lower sensitivity of phage L to the LT restriction.

Chromosomal location of the s system. Since recombinants which inherited the κ or κ *hsp* genes lost the *hspS* genes, it is most likely that both sets of genes have the same chromosomal location, that is, one minute on the left of the marker *thr*. To confirm this a *thr leu proC*

Table 2. Restriction-deficient mutants in the s system of *Salmonella typhimurium*

The mutants from experiments 1 to 3 were isolated among colonies infected by phage after plating about 1×10^3 cells from nitrosoguanidine treated cultures with 1×10^5 particles of Lc. LT. The mutants from experiments 4 to 7 were isolated among colonies which had inherited an F-lac⁺ from *Escherichia coli*.

Experiment number	Strain	Number of mutants with <i>hspS</i> phenotype	
		$r_s^-m_s^-$	$r_s^-m_s^+$
1	4233 LT7 $r_{LT}^+m_{LT}^+$	3	2
2	4214 LT2 $r_{LT}^+m_{LT}^+$	1	0
3	4274 LT2 $r_{LT}^+m_{LT}^+$	4	4
4	4109 LT7 $r_{LT}^+m_{LT}^+$	0	1
5	4253 LT7 $r_{LT}^-m_{LT}^-$	3	1
6	4256 LT7 $r_{LT}^-m_{LT}^+$	0	1
7	4247 LT2 $r_{LT}^-m_{LT}^+$	0	1

hspS hspLT derivative of LT7 was constructed and used as recipient in a cross with Hfr $\kappa 4$ which transfers the chromosome in the order O-*thr-leu-pro*. The inheritance of *hspS*⁺ was scored among various classes of recombinants selected in the *thr-leu* region of the map and compared to that of *hspLT*⁺ and *proC*⁺. It was found (Table 4) that recombinants which inherited *thr*⁺ but not *leu*⁺ from the donor inherited *hspS*⁺ at about the same frequency as *thr*⁺*leu*⁺ recombinants whereas recombinants which inherited *leu*⁺ but not *thr*⁺ inherited *hspS*⁺ much less frequently. Since the linkage of *thr* to *leu* was higher (94%) than that of *hspS* to *leu* (60%) it can be concluded that *hspS* map between the origin of Hfr $\kappa 4$ and *thr*.

The allelism of *hspK* and *hspS* was further demonstrated in two crosses between Hfr $\kappa 4$ and two recombinants of LT7 which inherited $r_{\kappa}^{-}m_{\kappa}^{+}$ and *ara* or *pyrA* from *Escherichia coli* (Colson & Colson, in preparation). These strains were chosen for two reasons. First, since they lacked κ restriction but had the κ modification ability it was possible to score the presence of the κ system in recombinants and eliminate the restriction of *Salmonella typhimurium* DNA in conjugation. Secondly, the markers *ara* and *pyrA* map between *thr* and *leu* and thus are linked to *hspS* and *hspK*. By selecting *ara*⁺ and *pyrA*⁺ recombinants and analysing their host specificity phenotypes it was possible to see whether κ modification was always lost when *hspS*⁺ was inherited from Hfr $\kappa 4$ or if recombinants could be obtained which had both systems or neither. Among 89 *ara*⁺ and 92 *pyrA*⁺ recombinants examined

Table 3. *s* and LT restrictions and modifications of phages P22 and L

The restriction coefficients are expressed as the quotient of the number of plaques counted on the strain exercising no restriction (4295) divided by the number of plaques counted on each restricting host. All strains are derivatives of LT7 *proC*90 (4233).

Phage	Restriction coefficient on strain			
	4295	4253	4423	4233
	$r_{LT}^{-}m_{LT}^{-}r_s^{-}m_s^{-}$	$r_{LT}^{-}m_{LT}^{-}r_s^{+}m_s^{+}$	$r_{LT}^{+}m_{LT}^{+}r_s^{-}m_s^{-}$	$r_{LT}^{+}m_{LT}^{+}r_s^{+}m_s^{+}$
P22c2.O	1	1.4	10,000	21,700
P22c2.K	1	2.5	21,000	62,000
P22c2.B	1	1.7	15,000	24,000
P22c2.S	1	1	8,000	12,000
P22c2.LT	1	1.6	1	1.6
P22c2.S,LT	1	1	1	1
Lc.O	1	66	2,000	23,000
Lc.K	1	100	3,100	43,000
Lc.B	1	83	2,800	31,000
Lc.S	1	1	1,100	2,200
Lc.LT	1	90	1	120
Lc.S,LT	1	1	1	1

Table 4. Inheritance of *hspS*⁺, *hspLT*⁺ and *proC*⁺ among recombinants between *Salmonella typhimurium* Hfr and F⁻ selected in the *thr leu* region

thr⁺ and *leu*⁺ Recombinants were selected in a cross between Hfr $\kappa 4$ and strain 4441 *thr leu proC hspLT hspS*. A large number of recombinants was first classified for the inheritance of *leu*⁺ and *thr*⁺. About 50 recombinants of each class were scored for the inheritance of the unselected markers.

Class of recombinant	Unselected marker (%)		
	<i>hspS</i> ⁺	<i>hspLT</i> ⁺	<i>proC</i> ⁺
<i>thr</i> ⁺ <i>leu</i> ⁺	60	23	25
<i>thr</i> ⁺ <i>leu</i>	54	4	2
<i>thr leu</i> ⁺	21	30	24

(Table 5) only donor and recipient type host specificity was observed. Hence it is assumed that *hspS* and *hspK* are allelic or at least very closely linked.

The s system in Escherichia coli. When restriction of incoming DNA was eliminated the exchange of *hspS* and *hspK* between *Escherichia coli* and *Salmonella typhimurium* was easy to obtain in *S. typhimurium* recipients either by mating *S. typhimurium* LT7 *mut* with *E. coli* Hfr (Colson & Colson, in preparation) or by mating $r_{\bar{K}}^- m_K^+$ recombinants of *S. typhimurium* with an *S. typhimurium* Hfr (see Table 5). An attempt was made to obtain the reciprocal event, that is, the introduction of the *s* system into *E. coli* by mating an *S. typhimurium* Hfr with a restriction-deficient mutant of *E. coli* K12. However, in this cross, between *hspS*⁺ Hfr K4 and *E. coli* the selected *thr*⁺*leu*⁺ recombinants were found at a very low frequency (about 10⁻⁷). After single colony reisolation, 40 of them were further analysed. All were sensitive to the male-specific phage MS2 and 15 of them restricted phages λ. K and λ. B. It was therefore assumed that they were, in fact, heterogenotes carrying F-*thr*⁺*leu*⁺ factors from Hfr K4 and that some of them had in addition the genes for the *s* system. This conclusion was confirmed by the observation that one of them transferred *thr*⁺*leu*⁺ together with the *s* system to both *E. coli* and *S. typhimurium* strains. In an *S. typhimurium* *thr leu* $r_{\bar{S}}^- m_S^+$ recipient, the transfer of *thr*⁺*leu*⁺ was accompanied by *s* restriction in all MS2-sensitive recombinants thought to have received the whole F-prime factor and in half of the MS2-resistant recombinants which had integrated the *thr*⁺*leu*⁺ part of it. After the transfer of the F-*thr*⁺*leu*⁺*hspS*⁺ factor to an $r_{\bar{B}}^- m_B^+$ strain, one recombinant was MS2 resistant and lacked B modification ability but inherited the *s* system. This recombinant remained stable in respect of the *s* restriction and modification and had most probably integrated the *hspS* genes in place of its original *hspB* genes. The stability of this strain permitted precise measurement of the action of the *s* system on the e.o.p. of phage λ. It was found (Table 6) that in *E. coli* the restriction coefficient of the *s* system on phage λ was much higher than that on phages P22 and L in *S. typhimurium* and was even higher than those of the K and B systems.

Table 5. Host specificity in recombinants from crosses between *Salmonella typhimurium* Hfr and strains with the K system of *Escherichia coli*

Derivatives of LT7 *mut* which had inherited *mut*⁺*leu*⁺*hspK* and either *ara* or *pyrA* from *E. coli* were crossed with Hfr K4; *ara*⁺ and *pyrA*⁺ recombinants were selected, restreaked on the selective media and scored for their restriction and modification phenotypes.

Recipient strain	Selected marker	Number of recombinants with phenotype	
		$r_{\bar{K}}^- m_K^+$	$r_S^+ m_S^+$
4321 <i>ara</i> $r_{\bar{K}}^- m_K^+$	<i>ara</i> ⁺	32	57
4328 <i>pyrA</i> $r_{\bar{K}}^- m_K^+$	<i>pyrA</i> ⁺	13	79

Table 6. K, B and *s* restrictions and modifications of phage λ

The restriction coefficients were calculated as in Table 3. The test strains were derivatives of *Escherichia coli* K12 and B. Strain 2026 has the *hspS*⁺ genes from *Salmonella typhimurium*.

Phage	Restriction coefficient on strain			
	1209 $r_{\bar{K}}^- m_K^+$	1200 $r_K^+ m_K^+$	2000 $r_B^+ m_B^+$	2026 $r_S^+ m_S^+$
λ. O	1	1,800	6,000	100,000
λ. K	1	1	2,200	5,500
λ. B	1	1,400	1	36,000
λ. S	1	2,500	5,000	1

DISCUSSION

Salmonella typhimurium exercises two independent host specificity systems of DNA restriction and modification. Since both systems are present in LT7 and LT2, it was not possible to detect their activities by transfer of DNA between these strains. However, they could be identified by means of recombinants between *Escherichia coli* and *S. typhimurium* LT7 *mut* which lacked the genetic determinants for them while still remaining sensitive to the Salmonella phages P22 and L. Mutations in the Salmonella host specificity systems could then be characterized and mapped in *Salmonella* × *Salmonella* crosses.

One of these host specificity systems, which we have designated LT (Colson & Colson, 1967) was first detected by Zinder (1960). It is governed by genes (*hspLT*) situated near *pro* (Colson *et al.* 1970) and has no known equivalent in *Escherichia coli*. We have shown that the second system, discovered only recently (Colson & Colson, in preparation) is governed by genes (*hspS*) situated at the same locus as the *hspK* and *hspB* genes in *E. coli* K12 and B. It is thus likely that the K, B and S systems together with the A system, which occupies this chromosomal locus in *E. coli* 15T⁻, (Arber & Wauters-Willems, 1970), evolved from a common ancestor and differ principally in the sequence of nucleotides which they each recognize on DNA.

Complementation studies between temperature-sensitive mutants in the *hsp* genes of *Escherichia coli* K12 and B strongly suggest that the products of these genes act as oligomeric enzymes (Hubacek & Glover, 1970). Since complementation studies also showed that some mutations in the B system could be complemented by the equivalent gene product of the K system and vice versa (Boyer & Roulland-Dussoix, 1969; Glover, 1970), the polypeptides coded by the *hspK* and *hspB* genes can interact to constitute functional enzymes. Whether the polypeptides coded by the *hspS* genes, present in a less closely related organism, have the capacity to interact with the *hsp* gene products of the systems studied in *E. coli* is an open question. The work described here provides nearly all the tools necessary for such a study which is now in progress.

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