Expression of the *Escherichia coli* κ , β and Phage P1 DNA Host Specificities in *Salmonella typhimurium*

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SUMMARY

The *Escherichia coli* κ , β and phage P1 host specificity genes (*hsp*) were introduced into *Salmonella typhimurium* by means of *E. coli* Hfr $\times$ *S. typhimurium* F⁻ crosses. The three systems were found to govern modification and restriction of phages P22 and L. The restriction coefficients were comparable to that of λ for the P1 system and were lower (about 100) for the κ and β systems. Phage grown on *hspK*⁺ and *hspB*⁺ recombinants was restricted by *S. typhimurium* independently of the restriction governed by the LT system. This observation suggested that *S. typhimurium* possesses a previously undetected *hsp* locus allelic to those of *E. coli* κ and β .

INTRODUCTION

Strains LT7 and LT2 of *Salmonella typhimurium* exercise specific control over the acceptance of foreign DNA and confer specific modification to DNA through mechanisms similar to those in *Escherichia coli* which have been reviewed by Arber & Linn (1969). In *S. typhimurium* the genetic determinants for these functions (*hspLT*) were mapped at a chromosomal location near to *proC* (Colson, Colson & Van Pel, 1970) and thus differ in map position from the genes controlling similar functions in *E. coli*, which are situated 1 min to the left of *thr* (Boyer, 1964; Colson, Glover, Symonds & Stacey, 1965; Wood, 1966).

To gain more information concerning host specificity in *Salmonella typhimurium* we have constructed *S. typhimurium* derivatives carrying various *Escherichia coli* *hsp* genes by means of crosses between *E. coli* Hfr and *S. typhimurium* LT7 *mut* (mutator gene) from which haploid recombinants can be isolated at a much higher frequency than with other *S. typhimurium* recipient strains (Colson & Colson, 1967). It was found that the κ , β and phage P1 host specificity systems can be detected using the salmonella phages P22 and L and that *S. typhimurium* possesses a previously undetected host specificity system. A detailed account of the properties of this system will be published separately (Colson & Colson, 1971).

METHODS

Bacteria. The strains of *Escherichia coli*, *Salmonella typhimurium* and *E. coli* $\times$ *S. typhimurium* recombinants used in this work are listed in Table 1.

Bacteriophages. The phages used were clear plaque mutants of P22 and L. The host modification of a phage is denoted according to previous recommendations (Arber & Linn, 1969). The symbol o is used for a phage lacking any detectable modification.

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

Analysis of recombinants. Recombinants between *Escherichia coli* Hfr and *Salmonella*

typhimurium LT7 *mut* were obtained as described by Colson & Colson (1967). All recombinants were first streaked on minimal or EMB-lactose agar and clones segregating pale or minute colonies were not analysed further. In some crosses haploidy of recombinants was confirmed from the inheritance of an unselected recessive marker. Single colonies of purified recombinants were tested for unselected markers. The presence of the mutator gene was detected by the method of Kirchner & Rudden (1966). Restriction and modification phenotypes were tested as described by Colson, Colson & Van Pel (1970).

RESULTS

The inheritance and expression of κ host specificity in *Salmonella typhimurium* was demonstrated in *leu*⁺ and *lac*⁺ recombinants from two crosses between *Escherichia coli* Hfr and a derivative of LT7 *mut* (Table 2, crosses 1 and 2). The recipient strain, a mutant deficient in LT restriction was chosen to eliminate restriction of *E. coli* DNA following transfer. The two donors were derivatives of HfrC differing in host specificity genotype. When the donor was wild-type (*hspK*⁺), 20 % of the *leu*⁺ and 10 % of the *lac*⁺ recombinants restricted phage P22: phages P22.0 and P22.LT both showed approximately 100-fold reduction of efficiency of plating (e.o.p.) on them. On the contrary, when the donor was a restriction deficient mutant ($r_{\kappa}^{-}m_{\kappa}^{+}$) none of the recombinants restricted P22. However, some of them host-modified P22 so that it was able to grow efficiently on the restricting recombinants isolated in the first cross and thus expressed the $r^{-}m^{+}$ phenotype of this host

Table 1. *Bacterial strains*

Strain no.	Genotype*	Restriction and modification phenotype	Origin
<i>Escherichia coli</i> K-12			
1014	HfrH <i>thi pyrA</i>	$r_{\kappa}^{+}m_{\kappa}^{+}$	—
1020	HfrH <i>thi hspK</i>	$r_{\kappa}^{-}m_{\kappa}^{-}$	Colson <i>et al.</i> (1965)
1027	HfrH <i>thi hspB</i> ⁺	$r_{\kappa}^{+}m_{\kappa}^{+}$	Glover & Colson (1969)
1021	HfrC <i>met</i>	$r_{\kappa}^{+}m_{\kappa}^{+}$	—
1033	HfrC <i>met ara hspK</i>	$r_{\kappa}^{-}m_{\kappa}^{+}$	From 1021
1306	HfrC <i>met</i> (P1 <i>cr</i>)	$r_{\kappa}^{+}m_{\kappa}^{+} r_{P1}^{+}m_{P1}^{+}$	Scott (1970)
<i>Salmonella typhimurium</i> LT7			
4012	<i>mut ara hspLT</i>	$r_{LT}^{-}m_{LT}^{+}$	From <i>hspLT82</i> (Colson & Colson 1967)
4016	<i>mut leu hspLT</i>	$r_{LT}^{-}m_{LT}^{-}$	From <i>hspLT1</i> (Colson & Colson 1967)
4233	<i>proC90</i>	$r_{LT}^{+}m_{LT}^{+}$	—
4253	<i>proC90 hspLT</i>	$r_{LT}^{-}m_{LT}^{-}$	Colson <i>et al.</i> (1969)
<i>Salmonella typhimurium</i> LT2			
4420	<i>met trp val str-r hspLT</i>	$r_{LT}^{-}m_{LT}^{+}$	Colson <i>et al.</i> (1969)
<i>E. coli</i> × <i>S. typhimurium</i> recombinants			
4301	(<i>leu</i> ⁺ <i>ara hspK</i>) ⁺ <i>mut</i>	$r_{\kappa}^{+}m_{\kappa}^{+} r_{LT}^{+}m_{LT}^{+}$	—
4304	(<i>mut</i> ⁺ <i>leu</i> ⁺ <i>ara hspK</i> ⁺) <i>hspLT</i>	$r_{\kappa}^{+}m_{\kappa}^{+} r_{LT}^{-}m_{LT}^{-}$	—
4321	(<i>mut</i> ⁺ <i>leu</i> ⁺ <i>ara hspK</i>) <i>hspLT</i>	$r_{\kappa}^{-}m_{\kappa}^{+} r_{LT}^{-}m_{LT}^{-}$	—
4329	(<i>mut</i> ⁺ <i>leu</i> ⁺ <i>ara</i> ⁺ <i>hspB</i> ⁺) <i>hspLT</i>	$r_{\kappa}^{+}m_{\kappa}^{+} r_{LT}^{-}m_{LT}^{-}$	—
4357	(<i>Lac</i> ⁺ <i>cam-r hspP1</i> ⁺ <i>tsx</i> ⁺) <i>met try val</i>	$r_{P1}^{+}m_{P1}^{+} r_{LT}^{-}m_{LT}^{-}$	—

* Markers in parentheses are those inherited from *Escherichia coli*.

specificity system. Therefore it was concluded that these recombinants had inherited the *hspK* genes from *E. coli* and exercised κ restriction and modification on the DNA of phage P22. This conclusion was confirmed by the results of crosses 3 and 4 (Table 2). In these experiments the recipient strain (4321) was a recombinant isolated in cross 2 (Table 2) with the *mut⁺leu⁺ara hspK* (r^-m^+) genes from *E. coli*. This recombinant was back-crossed to $r_K^+m_K^+$ and $r_K^-m_K^-$ derivatives of HfrH. As expected the donor host specificity phenotype was expressed in some of the selected *ara⁺* recombinants. In cross 5 (Table 2) recombinant 4321 was crossed with an *hsp B⁺* derivative of HfrH. Among *ara⁺* recombinants, 18 % were found to have lost the $r_K^-m_K^+$ phenotype and instead possessed another restriction and modification system effective on P22. Thus it appears that the *E. coli* B host specificity is also effective on P22 DNA in *S. typhimurium*.

The introduction of the *hspP_I* genes into *Salmonella typhimurium* was made possible with an *Escherichia coli* strain kindly given to us by Dr J. R. Scott. This strain is a derivative of HfrC in which a cryptic prophage (P_Icry) is integrated near the *lac* operon (Scott, 1970). This P_Icry contains part of the phage P_I genome, including the *hspP_I* genes, and a marker conferring resistance to chloramphenicol (*cam-r*). It was obtained by Scott from a recombinant between phage P_I and an R factor, the P_IdCM of Kondo & Mitsuhashi (1966). Since P_Icry is integrated near *lac* inheritance of *hspP_I* was looked for among *lac⁺* recombinants from a cross between HfrC (P_Icry) and an $r_{LT}^-m_{LT}^+$ derivative of *S. typhimurium* LT7 *mut*. With this recipient strain it was possible to score for the presence of the *hspLT* locus among the recombinants and ensure that donor DNA was not restricted during conjugation. Among 94 *lac⁺* recombinants (cross 6, Table 2) 72 reduced the e.o.p. of P22.0 and P22.LT by a factor of about 10^4 and they were $r_{LT}^-m_{LT}^-$. All but one of these recombinants also inherited *cam-r*. Thus *hspP_I* and *cam-r* are frequently inherited together with *lac⁺* and replace the *hspLT* locus. Nevertheless, 11 % of the *lac⁺* recombinants lost the m_{LT}^+ phenotype and did not inherit *hspP_I⁺*. Thus recombination can occur between the *hspP_I* and *hspLT* loci.

Table 2. *Inheritance of the κ , B, and phage P_I host specificities among recombinants between Escherichia coli Hfr and Salmonella typhimurium LT7 mut.*

The crosses were performed by mixing equal volumes of exponentially growing cultures of the parents for 1 h at 37° without shaking. The recombinants were selected on appropriate minimal media, purified by single colony re-isolation and scored for restriction and modification phenotype. The recipient strain 4321 in crosses 3, 4 and 5 is a recombinant from cross 2 which inherited *mut⁺leu⁺ara r_K⁻m_K⁺* from *E. coli*.

Cross no.	Donor	Recipient	Selected marker	Number of recombinants tested	No. of recombinants with host specificity phenotype indicated
1	1021HfrC $r_K^+m_K^+$	4016	<i>lac⁺</i>	99	11 $r_K^+m_K^+$
		$r_{LT}^-m_{LT}^-$	<i>leu⁺</i>	100	20 $r_K^+m_K^+$
2	1033HfrC $r_K^-m_K^+$	4016	<i>lac⁺</i>	99	9 $r_K^-m_K^+$
		$r_{LT}^-m_{LT}^-$	<i>leu⁺ara</i>	78	11 $r_K^-m_K^+$
3	1014HfrH $r_K^+m_K^+$	4321 $r_K^-m_K^+$	<i>ara⁺</i>	99	21 $r_K^+m_K^+$
4	1020HfrH $r_K^-m_K^-$	4321 $r_K^-m_K^+$	<i>ara⁺</i>	100	6 $r_K^-m_K^-$
5	1027HfrH $r_B^+m_B^+$	4321 $r_K^-m_K^+$	<i>ara⁺</i>	100	18 $r_B^+m_B^+$
6	1306HfrC $r_{P1}^+m_{P1}^+$	4012	<i>lac⁺</i>	96	11 $r_{LT}^-m_{LT}^-$
		$r_{LT}^-m_{LT}^-$			72 $r_{LT}^-m_{LT}^-r_{P1}^+m_{P1}^+$

Efficiency of plating of phages P22 and L on strains with the κ , B and P1 host specificity systems

The sensitivity of phages P22 and L to the κ , B, P1 and LT restrictions were studied by measuring e.o.p. of the phages on the appropriate *Escherichia coli* $\times$ *Salmonella typhimurium* recombinants as well as on wild-type *S. typhimurium* and also on a $r_{LT}^-m_{LT}^-$ mutant. The *hspK*⁺ and *hspB*⁺ test strains were chosen from among recombinants which had inherited *mut*⁺ from *E. coli* because cultures of strains with the *mut* phenotype usually contain enough *hsp* mutants to reduce significantly their restriction coefficients. This choice was not possible for the $r_K^+m_K^+$ $r_{LT}^+m_{LT}^+$ strain which was isolated only once in a cross between *E. coli* Hfr and LT7 *mut hspLT*⁺ and retained the *mut* phenotype. For the same reason the *hspPr*⁺ test strain was not one of the recombinants described in the previous section but a recombinant isolated from a cross between HfrC (P1*cry*) and a $r_{LT}^-m_{LT}^+$ derivative of LT2 (strain 4420) which yielded *lac*⁺ m_{LT}^- *hspPr*⁺ *cam-r* recombinants similar to those found with LT7 *mut*, but at a much lower frequency (10^{-7} instead of 10^{-4}).

The restriction coefficients in Table 3 show that phages P22 and L are both sensitive to the three systems from *Escherichia coli*. The r_{P1}^+ restriction coefficient was much higher than the r_K^+ and r_B^+ restriction coefficients for phages P22 and L and was about the same as the r_{P1}^+ restriction coefficient for phage λ in *E. coli* (P1). The r_K^+ and r_B^+ restriction coefficients for phages P22 and L were lower in these recombinants than they are for phage λ in *E. coli* κ and B. An unexpected observation was that the e.o.p. of P22.K, P22.B, L.K and L.B was reduced on *Salmonella typhimurium* $r_{LT}^-m_{LT}^-$ previously considered to lack restriction and modification. For P22 this reduction in e.o.p. was slight but for L it was about 100-fold. It was not due to the presence of an *E. coli* modification which the phages carried because it was observed with phage produced by $r_K^-m_K^-$ recombinants from cross 4, Table 2. In contrast, phage from *leu*⁺*ara* recombinants which had not inherited *hspK*⁺ from *E. coli* showed no such reduction of e.o.p. on *S. typhimurium*. The most plausible interpretation of these observations is that *S. typhimurium* possesses a host specificity system governed by genes situated at the same locus as the *hsp* genes in *E. coli*. The only strains lacking the capacity to confer this *S. typhimurium* modification and thus permitting detection of this system are those recombinants which inherited the *hsp* genes from *E. coli*.

Table 3. LT, K, B, and P1 restriction and modification of phages P22 and L

Lysates of phages P22 and L produced by each strain were suitably diluted and plated in overlays with exponentially growing cultures of the strains. The restriction coefficient for each phage is expressed as the quotient of the e.o.p. on the strain on which the lysate was produced divided by the e.o.p. on each host. Each value is based on a count of several hundred plaques.

Phage	Restriction coefficient on strain					
	4253 $r_{LT}^-m_{LT}^-$	4233 $r_{LT}^+m_{LT}^+$	4301 $r_{LT}^+m_{LT}^+$ $r_K^+m_K^+$	4304 $r_{LT}^-m_{LT}^-$ $r_K^+m_K^+$	4329 $r_{LT}^-m_{LT}^-$ $r_B^+m_B^+$	4357 $r_{LT}^-m_{LT}^-$ $r_{P1}^+m_{P1}^+$
P22.o	1	12,500	62,000	260	260	29,000
P22.LT	1	1	200	330	480	35,000
P22.K	2.5	62,000	1,000	1	300	—
P22.B	1.7	24,000	38,000	150	1	—
P22.P1	1	28,000	16,000	100	500	1
L.o	1	2300	62,000	170	230	11,000
L.LT	1	1	100	170	210	14,000
L.K	100	43,000	500	1	140	—
L.B	66	31,000	36,000	230	1	—
L.P1	1	1600	25,000	200	750	1

DISCUSSION

Analysis of recombinants between *Escherichia coli* and *Salmonella typhimurium* shows that the *E. coli* κ , β and phage P1 host specificity systems are effective on the DNA of the salmonella phages P22 and L. This means that the base sequences recognized by these restriction enzymes are present at least once on the DNA of these phages. Within limits the restriction coefficient for a given phage by a given system is a measure of the number of sites present on the phage DNA (Franklin & Dove, 1969). Phage λ , for example, which has a single site for the A system has a restriction coefficient of 100 (Arber & Wauters-Willems, 1970). Thus the restriction coefficient of 100 observed when P22 and L are subjected to either κ or β restriction may reflect the presence of only one restriction sensitive site for each system on their DNA. On the contrary the P1 restriction coefficient is much higher and probably reflects the presence of more than one site.

The most satisfactory explanation for the unexpected observation that phages P22 and L grown on recombinants with the κ or β *hsp* genes are restricted by *Salmonella typhimurium* independently of the activity of the LT system is that *S. typhimurium* possesses a restriction and modification system governed by genes situated at the same locus as the *hspK* and *hspB* genes in *Escherichia coli*. Recent experiments have confirmed this interpretation (Colson & Colson, 1971).

There is apparently no *hsp* locus in the *pro lac* region of *Escherichia coli* corresponding to the *hspLT* locus in *Salmonella typhimurium*. However, the possibility cannot be ruled out that such a system exists in all *E. coli* strains so far tested but has remained undetected because *E. coli* DNA is always modified in respect of it and thus never restricted upon transfer between *E. coli* strains. If such a system is present in *E. coli* then one must assume that the DNA of phages P22 and L have no sensitive site for it since recombinants with the *lac* region of *E. coli*, which would have inherited this system, did not restrict these phages. We have not obtained yet a derivative of *E. coli* carrying the *pro* region of *S. typhimurium*, such a recombinant would have lost this system and produce DNA with no modification at the appropriate sites for that restriction.

In the strain of *Escherichia coli* isolated by Scott (1970) the *hspP1* genes are integrated into the chromosome near *lac*. Thus the hypothesis might be considered that the LT system of *S. typhimurium* is located on the chromosome as the result of integration of an *hspLT* plasmid which might be related to P1 or to some other plasmid-controlled system in *E. coli*. Recombination between *hspP1* and *hspLT* shows that the map position of *hspP1* in the strain of Scott is close to but not allelic to that of *hspLT*. We have observed (A. M. Colson & A. Thirion, unpublished) that in reciprocal Hfr $\times$ F⁻ crosses between *E. coli* and *Salmonella typhimurium* the presence of this P1^{cry} in the *E. coli* strain does not promote an increased frequency of recombination in the *pro lac* region. Such an increase might have been expected to occur if the integration of this plasmid created a region of better synaptic homology between the chromosomes of these strains. These results suggest that the P1 and LT systems are genetically distinct. The functional relationship of the LT and the P1 systems remains to be examined in complementation experiments.

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