DNA Restriction and Modification Systems in *Salmonella*. * SQ, a New System Derived by Recombination Between the SB System of *Salmonella typhimurium* and the SP System of *Salmonella potsdam*

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SUMMARY

As the result of P1-mediated cotransduction with *serB* from *Salmonella potsdam* to the *Escherichia coli*/*Salmonella typhimurium* hybrid 4617, one recombinant, L4004, was isolated which had a restriction-modification (R-M) system different from the SB and SP systems of its parents, and was designated SQ. The genes of SQ were allelic to those of the SB system of *S. typhimurium* and were shown by complementation experiments to be functionally related to those of the K system of *E. coli*. Evidence that the SQ system in L4004 arose as the result of a recombination event within the *hsdS* genes of SB and SP is discussed.

INTRODUCTION

Restriction enzymes are currently classified into two types depending on their properties (for a recent review, see Arber, 1974). Enzymes of type I contain three types of subunits, have complex cofactor requirements and undergo complex interactions with DNA. Most known restriction-modification systems (R-M systems) with type I enzymes are coded by genes on the bacterial chromosome. These genes have the remarkable property of occupying an allelic locus in different bacterial strains. Thus the genes for the *Escherichia coli* K, B and A systems as well as those of the *Salmonella typhimurium* SB and *Salmonella potsdam* SP systems map close to and counter-clockwise to *serB* (Boyer, 1964; Glover & Colson, 1969; Arber & Wauters-Willems, 1970; Colson & Van Pel, 1974; Bullas & Colson, 1975a). In addition, complementation studies in merodiploids have indicated that three of these systems have functional homology – the K, B and SB systems (Arber & Linn, 1969; Boyer & Roulland-Dussoix, 1969; Glover, 1970; Van Pel & Colson, 1974). It is conceivable that these R-M systems were derived from a common ancestor. From the present state of knowledge of restriction enzymes and their genetic determinants, one can suggest that these different R-M systems resulted from alterations in the *hsdS* gene, coding for specific site recognition so that its product then recognized different nucleotide sequences.

The possibility of producing new R-M systems by mutations in *hsdS* genes or by recombination within *hsdS* genes has been suggested by Arber & Linn (1969). However, no new experimentally-produced R-M system with an altered specificity has yet been reported.

In a previous paper we demonstrated allelism between the SB system of *S. typhimurium*

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Table 1. Strains of bacteria

Strain no.	Host specificity phenotype	Genotype*	Reference†
<i>Escherichia coli</i> K12			
1225	r_K^+ m_K^+	<i>thr leu thi lac</i>	1
1228	r_K^- m_K^-	<i>thr leu thi lac hsdS_K</i>	1
1240	r_K^- m_K^+	<i>thi serB str^r hsdR_K</i>	1
<i>Salmonella typhimurium</i> LT2			
4247	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>metA metB trpB str^r</i>	2
4419	r_{LT}^- m_{LT}^+ r_{SA}^- m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>metA metB trpB val str^r hsdR_{LT} hsdR_{SA}</i>	2
4514	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>serB80 galE</i>	2
4519	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>serA13 thr-17</i>	2
4532	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>metA metB trpB val pyrB124 gal</i>	2
4536	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>metA metB trpB val serB80 gal</i>	2
<i>Escherichia coli</i> /S. <i>typhimurium</i> hybrid			
4617	r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+	<i>thi lac⁻ (serB80 leu⁺)St</i>	1
<i>Escherichia coli</i> /S. <i>typhimurium</i> /S. <i>potsdam</i> hybrids			
L4001	r_{SA}^- m_{SA}^- r_{SB}^+ m_{SB}^+	<i>thi lac (leu⁺)St (serB80⁺ hsd_{SA}⁻)^{Sp}</i>	2
L4002	r_{SA}^- m_{SA}^- r_{SP}^+ m_{SP}^+	<i>thi lac (leu⁺)St (serB80 hsd_{SA}⁻)^{Sp}</i>	2
L4003	r_{SA}^+ m_{SA}^+ r_{SP}^+ m_{SP}^+	<i>thi lac (leu⁺)St (serB80 hsd_{SA}⁻)^{Sp}</i>	2
<i>Salmonella typhimurium</i> /S. <i>potsdam</i> hybrids			
L3001	r_{LT}^+ m_{LT}^+ r_{SA}^- m_{SA}^- r_{SB}^+ m_{SB}^+	<i>metA metB trpB val (pyrB⁺ hsd_{SA}⁻)^{Sp}</i>	2
L3002	r_{LT}^+ m_{LT}^+ r_{SA}^- m_{SA}^- r_{SP}^+ m_{SP}^+	<i>metA metB trpB val (pyrB⁺ hsd_{SA}⁻)^{Sp}</i>	2
L3003	r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SP}^+ m_{SP}^+	<i>metA metB trpB val (pyrB⁺ hsd_{SA}⁻)^{Sp}</i>	2

* Genes outside brackets are from the 'majority parent'; genes inside brackets are from minority parent(s). The superscripts St and Sp designate genes from *S. typhimurium* and *S. potsdam* respectively.

† References: 1, Colson & Van Pel (1974); 2, Bullas & Colson (1975a).

and the SP system of *S. potsdam* (Bullas & Colson, 1975a). During the course of these experiments, we found an exceptional recombinant with a new R-M system. This paper reports the results of genetic experiments with this recombinant which support the interpretation that it arose as the result of recombination within the *hsdS* genes of the SB and the SP systems. A preliminary report of our results has already been published (Bullas & Colson, 1975b).

METHODS

Bacteria and bacteriophages. The various strains of *Escherichia coli*, *Salmonella typhimurium*, *E. coli*/Salmonella and Salmonella/Salmonella hybrids are listed in Table 1. *Escherichia coli* phages P1, P2, 82 and 434 were from our own collection. Phage λ , phage P3 which lysed both *E. coli* K12 and various strains of Salmonella, and *S. typhimurium* phages P22 and L were as used previously (Bullas & Colson, 1975a).

Notations. The notations for host specificity phenotypes and genotypes, and for phage modifications are the same as those used previously (Bullas & Colson, 1975a) and follow the recommendations of Arber & Linn (1969) and Arber (1974).

Transductions. Transductions were with phage P1, in the manner previously described (Bullas & Colson, 1975a).

Table 2. *Efficiencies of plating of P₃ with different modifications on strains with the SB, SP and SQ specificities*

Strain	R-M system	Efficiency of plating of phage*			
		P ₃ .O	P ₃ .SB	P ₃ .SP	P ₃ .SQ
L4001	SB	1.7×10^{-2}	0.79	2.5×10^{-2}	2.7×10^{-2}
L4002	SP	2.5×10^{-3}	4.8×10^{-3}	1.0	1.1×10^{-2}
L4004	SQ	1.9×10^{-2}	8.6×10^{-3}	2.2×10^{-2}	0.34

* The efficiencies of plating recorded are relative to that on *E. coli* K12 strain 1228.

Table 3. *P₁-mediated cotransduction of hsd_{sq} with $serB$ in *E. coli*/S. *typhimurium* hybrid 4617*

Donor strain	Recipient strain	Number of Ser B ⁺ recombinants with:				Total
		hsd_o	hsd_{sb}^+	hsd_{sq}^+	$hsd_{sb\ sq}^+$	
L4004	4617	0	150	102	0	252

Conjugations. Crosses between *S. typhimurium* Hfr and *E. coli* F⁻, and characterization of hybrids were done as described by Colson & Van Pel (1974).

Measure of efficiency of plating of phages. The efficiency of plating (e.o.p.) was indicated by the number of plaques on the test strain relative to the number of plaques on a strain with no detectable R-M system. An approximate estimate of the e.o.p. was obtained by spotting known dilutions of phages on the same strains and was adequate, for example, to determine the restriction and modification phenotype of recombinants.

Single-step growth curves. The method of Adams (1959) was followed. Generally, the multiplicity of infection was about 0.01. Samples from the growth tubes were plated out with strain 1228 which possessed zero restriction and modification.

RESULTS

Isolation of recombinant L4004 with a new restriction-modification phenotype

By means of P₁ transduction from *S. potsdam* to the *E. coli*/S. *typhimurium* hybrid 4617 we have demonstrated that the SB system present in *S. typhimurium* LT2 is allelic to the SP system in *S. potsdam* (Bullas & Colson, 1975a). No recombinants have ever been isolated that possessed both systems. However, from among a total of 230 SerB⁺ recombinants that were tested, one recombinant was found which failed to restrict either λ.SB or λ.SP. This recombinant was designated L4004.

Modification tests with phage λ indicated that L4004 failed to modify for either the SB or the SP specificities. This recombinant therefore had the phenotype $r_{sb}^- m_{sb}^- r_{sp}^- m_{sp}^-$. Thus L4004, an unusual recombinant which had lost the specificity of one parent without acquiring the specificity of the other, was investigated further.

Efficiency of plating of E. coli phages on L4004

The possibility that L4004 had an R-M system but that λ had no recognition site for this system was investigated by measuring the e.o.p. of a number of other *E. coli* phages on this recombinant. Phages P₁, P₂, 82, 434 and P₃ were available from our own collection and were therefore used. Results of these tests indicated that phage P₁ was not restricted by strains with the SB or the SP systems, nor by L4004; phages λ, P₂, 82 and 434 were each

Table 4. Efficiencies of plating of phages P22 and L with different modifications, on strains of *S. typhimurium* with the SB, SP and SQ restrictions

Strain no.	Restriction phenotype	Efficiency of plating of phage*					
		P22.LT,SA, SB	P22.LT,SA, SP	P22.LT,SA, SQ	L.LT,SA, SB	L.LT,SA, SP	L.LT,SA, SQ
4247	$r_{LT}^+ r_{SA}^+ r_{SB}^+$	1.0	0.84	0.76	1.0	0.15	0.82
L3003	$r_{LT}^+ r_{SA}^+ r_{SP}^+$	1.8×10^{-3}	1.0	2.1×10^{-3}	1.4×10^{-3}	1.0	2.3×10^{-3}
L3004	$r_{LT}^+ r_{SA}^+ r_{SQ}^+$	6.7×10^{-4}	3.8×10^{-3}	1.0	9.1×10^{-4}	1.4×10^{-3}	1.0

* The efficiencies of plating recorded are relative to that on the corresponding propagating strain for each of the phages.

restricted by strains with the SB and the SP systems but were not restricted by L4004; phage P3, on the other hand, was restricted by L4004 as well as by strains with the SB and the SP systems.

A detailed e.o.p. analysis of P3 on strains with the SB and the SP systems as well as on L4004 (Table 2) indicated that L4004 has an R-M system, which we have called SQ, whose specificity is different from that of either SB or SP.

That the reduced efficiencies of plating of P3 on L4004 shown in Table 2 were due to a restriction-modification mechanism was more directly indicated from the results of a series of single-step growth experiments of P3 with various modifications on L4004. P3 propagated on L4004 (i.e. P3.SQ) gave the expected normal growth response, with lysis commencing 50 min after adsorption at 37 °C and an average burst size of 47. On the other hand, no lysis of L4004 occurred with either P3.SB (propagated on strain 4617) or P3.O (propagated on strain 1228). Only at a high multiplicity of infection of 3 was there lysis with P3.O with an average burst size of only 6. P3.O,SQ (P3.O propagated on L4004) gave single-step growth parameters that were similar to those obtained with P3.SQ while no lysis of L4004 occurred with P3.SQ,O (P3.SQ propagated on 1228). These results indicated that P3 without the SQ modification was restricted by L4004 and supported the interpretation that the reduced efficiencies of plating of P3 on L4004 were due to a restriction-modification mechanism.

*Test for allelism of hsd_{sq} to hsd_{sb} in *E. coli*/*S. typhimurium* hybrid 4617*

To map the genes of the new SQ system, a P1 lysate was prepared on L4004 and used to transduce *serB*⁺ to hybrid 4617. *SerB*⁺ recombinants were examined for their R-M phenotypes. The results are summarized in Table 3. Of 252 *SerB*⁺ recombinants examined, 102 or 40 % inherited the $r_{sq}^+ m_{sq}^+$ phenotype. Thus SQ is controlled by chromosomal genes linked to *serB*. Moreover, no recombinant was found that lacked both the SB and the SP restrictions or expressed both restrictions. Thus hsd_{sq} behaved as an allele of hsd_{sb} . This result implies that hsd_{sq} is also an allele of hsd_{sp} since hsd_{sp} is an allele of hsd_{sb} (Bullas & Colson, 1975a).

*Sensitivity of *S. typhimurium* phages P22 and L to hsd_{sq}*

Hsd_{sq} was introduced into *S. typhimurium* strain 4536₁ by P1 cotransduction with *serB* from L4004. Of 39 *SerB*⁺ recombinants isolated, four had lost the SB phenotype and acquired the SQ phenotype as detected with the use of phage P3. In addition, these four SQ⁺ recombinants restricted both phages P22 and L. One recombinant was selected and designated L3004. A detailed e.o.p. analysis of the restriction of P22 and L, each with the SB,

SP and SQ modifications, by *S. typhimurium* strains 4247 (SB⁺), L3003 (SP⁺) and L3004 (SQ⁺) was carried out. The results (Table 4) show the insensitivity of P22 and L to SB restriction (Colson & Van Pel, 1974) but clearly show that they are sensitive to SP and SQ restrictions. These results confirm the conclusion previously drawn that L4004 was a recombinant which had acquired a new R-M system with a specificity different from that of either of the parental SB or SP systems.

Complementation of an hsdR_K mutation by hsd_{sq}

To demonstrate further that the SQ system belongs to the series of related systems with type I enzymes, evidence of functional relatedness of SQ with the K system of *E. coli* was investigated.

An *hsd_{sq}⁺* derivative of *S. typhimurium* HfrK4 was first obtained in the following manner. Strain 4519 was made sensitive to phage P1 by selecting a rough *gal* spontaneous mutant (Ornellis & Stocker, 1974; Mojica-a, 1975). This *gal* strain was made *serA*⁺ and then *serB*⁻ *thr*⁺ by two successive P1 transductions from the *serB*⁻, P1-sensitive strain 4514. Finally, the *serB*⁻ derivative of 4519 was made *hsd_{sq}⁺* by P1 cotransduction with *serB*⁺ from strain L3004.

The *hsd_{sq}⁺* HfrK4 strain was then used as donor in conjugation with *E. coli* strain 1240, a *serB hsdR_K* derivative of HfrH which had lost donor ability. When used as recipient in crosses with HfrK4, this strain yields an unusually high proportion of those hybrids which are diploid for the *serB hsd* region as a result of the integration of this region of the *S. typhimurium* chromosome at a non-homologous locus (Colson & Van Pel, 1974).

Among 11 such hybrids examined for their restriction capabilities, six showed SA restriction, SQ restriction and K restriction. To confirm that K restriction resulted from complementation of the *hsdR_K* mutation in the recipient chromosome, one of these hybrids was used to transduce *thr*⁺ to strain 1225. Among more than 100 *Thr*⁺ recombinants examined, 30 % had inherited *serB*⁻, indicating that the hybrid had retained the recipient *serB*⁻ mutation. In addition, 3 % of the *Thr*⁺ transductants had the *r_K⁻ m_K⁺* phenotype, indicating that the hybrid had retained the *hsdR_K* mutation of the recipient in spite of its *r_K⁺ m_K⁺* phenotype. Since it has been demonstrated that *hsd_K* is not complemented by *hsd_{sa}* (Van Pel & Colson, 1974), K restriction in this hybrid must have occurred as the result of complementation by *hsd_{sq}*.

DISCUSSION

Recombinant L4004 clearly has an R-M system, SQ, which is different from those of its parents. SQ did not originate from an uncontrolled event such as plasmid infection but was the result of a recombination event between *hsd_{sb}* and *hsd_{sp}* genes. Its genetic determinants were shown by P1 transduction to be chromosomally located and allelic to those of the SB and the SP systems. Moreover, SQ was shown by complementation experiments to be functionally related to the K system of *E. coli*. Thus SQ is another member of the multi-allelic series of related R-M systems in *E. coli* and *Salmonella* (Bullas & Colson, 1975a).

The most likely explanation for the origin of SQ is a cross-over within the *hsd_{sb}* and *hsd_{sp}* genes. Certainly, other possibilities, such as mutation in genes of the SB or SP systems during the process of recombination repair, cannot be ruled out but would seem to be less probable.

Active sites for SQ were present in the three *Salmonella* phages, P3, P22 and L, but were absent from all the *E. coli* phages tested. This ecological distribution of sites is probably without significance however, since *E. coli* phages, particularly phage λ, possessed both SB

and SP sites (Colson & Van Pel, 1974; Bullas & Colson, 1975a). Moreover, it should be remembered that L4004 was originally recognized because phage λ was unrestricted, i.e. recognition and selection of L4004 was made with an *E. coli* phage that specifically lacked SQ recognition sites. A survey of other *E. coli* phages, particularly those unrelated to λ , should certainly reveal some that possess SQ recognition sites.

During a subsequent systematic search for other L4004-like recombinants, only one more $hsd_{SB}^- hsd_{SP}^-$ recombinant was found among 1443 SerB⁺ recombinants examined. However, this recombinant was found to be unusual in other respects and its properties will be reported in a separate paper. Thus the overall frequency with which L4004 was found was no greater than 0.07 %. This frequency is at least 0.05 times the frequency at which wild-type *hsd* recombinants were observed (1.3 %) in similar crosses between mutants in the *hsdR* and *hsdS* genes (Bullas & Colson, 1975a).

Since the genes *hsdR* and *hsdS* are closely linked and since the frequency of recombination between mutations in these genes was observed in 1.3 % of SerB⁺ recombinants, it is likely that recombinational events within *hsdS* genes of different specificities (K, B, SB, SP) occur at a higher frequency than that which generated L4004, but remain undetected because the composite *hsdS* gene retains the specificity of one of the parents. One might expect that such *hsdS* genes would code for hybrid polypeptides unable to bind efficiently, or in the right configuration, with the other polypeptides so that no functional enzyme or even inactive enzyme would be formed. The fact that no such recombinant was observed in this work nor in earlier work (e.g., Glover & Colson, 1969; Bullas & Colson, 1975a) perhaps indicates that *hsdS* genes in different systems do not differ sensibly in that part of their sequence which determines the binding properties of the subunit.

This view would imply that *hsdS* genes for different systems are largely similar and that recombination events leading to a loss of specificity or a change in specificity, as in recombinant L4004, would need to occur in a small restricted region of the gene. This region of the *hsdS* gene could be that which codes for the active site allowing for the recognition of a nucleotide sequence by that subunit for the type I enzyme which is responsible for the site recognition.

As a result of the isolation of the SQ system in the laboratory and from the fact that multiple, active alleles of the *hsdS* gene exist in nature, one is led to speculate that this site has an amazing plasticity. Alterations in its amino-acid sequence as a result of recombination or, perhaps, mutation can produce a new active site, specific for another nucleotide sequence.

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