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Complementation and Hybridization Evidence for Additional Families of Type I DNA Restriction and Modification Genes in *Salmonella* Serotypes

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Of eight *Salmonella*, *serB*-linked *hsd* genes for the restriction and modification of DNA transferred to *Escherichia coli*/*Salmonella* hybrids, only two—those with SM and ST (*S. muenchen* and *S. thompson*, respectively) specificities—may have weakly complemented r_{SB}^- and none complemented r_K^-. An A-specific DNA probe failed to hybridize to *Hin*dIII-restricted fragments of each of the hybrids, but an SB (*S. typhimurium*)-specific probe hybridized to DNA from the hybrid with ST specificity. These results indicate that additional families of the type I *hsd* genes may exist.

Systems of genes determining the production of type I restriction endonucleases and modification methylases have been recognized both in *Escherichia coli* and *Salmonella* spp. (2, 5). In both genera, the genes coding for the enzymes are located on the chromosome and map close and counterclockwise to *serB* (1, 5, 21, 23). In transduction experiments using bacteriophage P1, all these systems behave as alleles either of the *E. coli* K system or of the SB system of *S. typhimurium*. That is to say, after recombination, only one of the systems is found to be present on the chromosome. No recombinant has yet been isolated in which two systems are present at the same time. In addition, two plasmid-associated type I systems have been recognized in *E. coli* (9, 19).

Genetic analyses of the *E. coli* K and B systems and the *S. typhimurium* SB system have indicated that each system encodes three genes (*hsd* for host specificity DNA): *hsdR*, *hsdM*, and *hsdS* in that order (3, 4, 13, 20). Only the product of *hsdS* is essential for the specificity, whereas the products of both *hsdS* and *hsdM* are necessary for modification and the products of all (*hsdS*, *hsdM*, and *hsdR*) are required for restriction (24). The genes of the *E. coli* A and E systems, which also behave as alleles of the *E. coli* K and the B systems of genes, are similarly constituted (11, 22). Furthermore, all systems investigated have a promoter upstream from *hsdR* for transcription of the genes for restriction and a second promoter between *hsdR* and *hsdM* for transcription of the genes for modification (11, 20, 22).

Perhaps the most distinctive biological property of the type I enzymes is their recognition of a hyphenated specificity sequence consisting of a domain of 3 nucleotides separated by a short nonspecific sequence from a second specific domain of 4 or 5 nucleotides. Eight such specificity sequences have now been recognized. The nucleotide sequences within the *hsdS* gene which correlate with the sequences recognized have been identified for some of these type I *hsd* genes (10, 12).

However, despite their similarities and their behavior as alleles in genetic recombination experiments, the *serB*-linked *hsd* systems of genes are members of more than one complementation group. Thus, the *hsd* genes of the *E. coli* systems with K, B, and D specificities and the *Salmonella* systems with SB (from *S. typhimurium*), SP (from *S. potsdam*), and SQ (a hybrid system derived by intragenic recombination between the *hsdSB* and the *hsdSP* genes) specificities constitute a single complementation group (3, 4, 6, 7, 12, 14). Immunological cross-reactivity tests using antisera against each of two subunits of the *E. coli* K system, as well as DNA hybridization between the different *hsd* genes as a measure of homology, provided additional molecular evidence that the *E. coli* K system was similar to the *Salmonella* SB and SP systems (18). For these reasons, these *E. coli* and *Salmonella* systems of *hsd* genes were designated a family by Fuller-Pace et al. (11).

The *E. coli hsdA* and *hsdE* genes constituted a second complementation group. Furthermore, these two systems of genes were shown to be similar on the basis of DNA homology and to be nonhomologous to *hsdK*. On the basis of these results, the *E. coli* A and E systems were designated a second family (11). A search for members of each of these two families among other members of the *Enterobacteriaceae*, using DNA hybridization to A- and K-specific probes as a measure of similarity, has recently been done (8).

Other *hsd* genes have been recognized in additional *Salmonella* serotypes (5). Some of these systems were transferred by cotransduction with *serB* to an *E. coli*/*S. typhimurium* hybrid in which the *S. typhimurium* LT2 *hsdSB* genes had replaced the *E. coli hsdK* genes. Phage lambda modified by propagation on hybrids with each of these *serB*-linked, *Salmonella* systems was restricted by *E. coli* 1225 (r_K^+), 2000 (r_B^+), and 2379 (r_A^+), indicating that the specificities of these *Salmonella* systems were different from those of these three *E. coli* systems (5). Thus, these other *Salmonella hsd* systems all behave as alleles of SB and are closely linked to *serB* and therefore probably constitute additional members of the *serB*-linked systems of *hsd* genes coding for type I DNA restriction and modification enzymes. However, we have demonstrated in this study that none of these other *Salmonella* restriction-modification (R-M) genes complemented the restriction gene of the *E. coli* K system and only one or two of them weakly complemented the restriction gene of the *S. typhimurium* SB system. Furthermore, with the exception of one of the systems which weakly complements r_{SB}^-, neither an A-specific nor an SB-specific probe hybridized to DNA from any of the hybrids. From these

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TABLE 1. Bacterial strains

Strain or hybrid	R-M phenotype[a]	Source or reference
E. coli		
1225	$r_K^+ m_K^+$	5
1228	$r_K^- m_K^-$	5
1315	$r_A^+ m_A^+$	C. Colson
2000	$r_B^+ m_B^+$	5
2379	$r_A^+ m_A^+$	C. Colson
C	None	This laboratory
JW407	$r_K^- m_K^+$	J. Wecksler
S. typhimurium LT2		
SA534 (HfrK4)	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	K. Sanderson
4247	$r_{LT}^+ m_{LT}^+ r_{SA}^+ m_{SA}^+ r_{SB}^+ m_{SB}^+$	5
E. coli K/*S. typhimurium* LT2 4662	$r_{SB}^+ m_{SB}^+$	5
E. coli K/*S. typhimurium* LT2/*Salmonella* sp.		
4001	$r_{SB}^+ m_{SB}^+$	4
4002	$r_{SP}^+ m_{SP}^+$	4
4020	$r_{SM}^+ m_{SM}^+$	4
4021	$r_{ST}^+ m_{ST}^+$	5
4023	$r_{SK}^+ m_{SK}^+$	5
4029	$r_{SEA}^+ m_{SEA}^+$	5
4030	$r_{SEN}^+ m_{SEN}^+$	5
4037	$r_{SBL}^+ m_{SBL}^+$	5
4039	$r_{SG}^+ m_{SG}^+$	5
4043	$r_{SBA}^+ m_{SBA}^+$	5

[a] Subscripts K, A, and B refer to *E. coli* K, A, and B specificities, respectively. LT refers to the specificity common to most *Salmonella* species, and SA refers to the unique specificity to *S. typhimurium* (5). SB refers to specificity to *S. typhimurium*, SP to *S. potsdam*, SM to *S. muenchen*, ST to *S. thompson*,, SK to *S. kaduna*, SEA to *S. eastbourne*, SEN to *S. enteritidis*, SBL to *S. blegdam*, SG to *S. gelsenkirchen*, and SBA to *S. bareilly*, all associated with *serB*-linked *hsd* genes.

results we conclude that there are additional families of the type I *hsd* genes.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. Bacterial strains are listed in Table 1. Phage P1 used for transductions and phages used for determining the R-M phenotypes are the same as those used by Bullas et al. (5).

Media and buffers. Media and buffers were also the same as those previously used (5).

Notation. Notations for host specificity genotypes and phenotypes are the same as used by Bullas et al. (5). In this paper, *hsd* followed by the symbol of the specificity (K, B, A, SB, etc.) is used as an abbreviation for the *hsd* genes (*hsdR*, *hsdM*, and *hsdS*) of the system indicated by the symbol.

Determination of R-M phenotypes. R-M phenotypes of bacteria were determined by estimating the efficiency of plating (EOP) of the indicator phages on the selected recombinants relative to the EOP on *E. coli* C, which lacks an R-M system. Since small decreases in the EOP could be due to differences in phage adsorption as well as to restriction, we interpreted EOPs between 0.5 and 1.0 as indicating no evidence of restriction, EOPs between 0.1 and 0.5 as indicating possible restriction, and EOPs less than 10^{-1} as due to restriction. Boyer and Roulland-Dussoix (3) found that complementation between *E. coli hsdK* and *hsdB* genes results in a much reduced level of restriction, since unmodified phages plate out with EOPs around 10^{-1} in complementation as compared with about 10^{-3} on strains with the wild-type genes.

Complementation tests. To test for complementation between the *hsdR* subunit of *E. coli hsdK* and other *Salmonella serB*-linked *hsd* genes, an F-prime plasmid with the $r_K^- m_K^+$ phenotype was constructed. F101 is an F-prime plasmid that carries the section of the *E. coli* chromosome which includes the *hsdK* genes (16), while *E. coli* JW407 has the $r_K^- m_K^+$ phenotype with a Tn*10* insertion close to the *hsdK* genes. We demonstrated that Tet^r was 31% cotransducible with $r_K^- m_K^+$. This Tn*10*-linked $r_K^- m_K^+$ allele of JW407 was transferred to F101 in *E. coli* 1228 ($r_K^- m_K^-$) by P1 transduction. This $r_K^- m_K^+$ F-prime plasmid was designated F101–102. The presence of the F-prime plasmid was confirmed with the use of phage MS2.

An F-prime plasmid carrying the 98- to 0-min of the *S. typhimurium* chromosome was also isolated. A *thr*::Tn*10* mutant was isolated from a random Tn*10* pool of the *S. typhimurium* LT2 chromosome obtained from G. Ames (University of California, Berkeley). This *thr*::Tn*10* mutation was then transferred by P22 transduction to the *S. typhimurium* HfrK4 strain SA534, which transfers the chromosome in the following order: origin-*pyrB-thr-leu-*. The *hsdSB* genes are located between *pyrB* and *thr*. *S. typhimurium* SA534 (which is Str^s) with this *thr*::Tn*10* mutation was then conjugated with a *recA* derivative of *E. coli* 1228 ($r_K^- m_K^-$) which is *ser leu* and is Str^r. Selection for *ser*$^+$ *leu*$^+$ Tet^r Str^r derivatives therefore selected for cells in which these genes had been recombined into a deintegrated F-prime plasmid (15).

Although the majority of these exconjugants quickly lost their F-prime plasmid, a stable $r_{SB}^- m_{SB}^+$ exconjugant was isolated. This derivative which was designated FS680-1 therefore possessed an F-prime plasmid with the genotype *ser*$^+$ *leu*$^+$ *thr*::Tn*10* *hsdR*$_{SB}$.

DNA hybridization tests. DNA probes were nick translated with ^{32}P as the radioactive label. Hybridizations were carried out by the methods of Maniatis et al. (17). Autoradiography was carried out at −70°C with Ortho G X-ray film and an intensifying screen.

RESULTS AND DISCUSSION

Complementation of *E. coli* restriction function. We demonstrated that the r_K^- carried on F101–102 was complemented by r_B^+ from *E. coli* and r_{SB}^+ and r_{SP}^+ from *S. typhimurium* and *S. potsdam*, respectively (3, 4). The EOPs of phage lambda with each of these eight *Salmonella* modifications were all between 10^{-2} and 10^{-4} on *E. coli* 1225 (r_K^+) and hybrid 4662 (r_{SB}^+), indicating restriction. On the other hand, no complementation was observed between r_K^- and any of the eight *Salmonella* chromosomal, *serB*-linked *hsd* genes in the other hybrids. That is to say, lambda phages modified with a single *Salmonella* modification (e.g., lambda with SEN [*S. enteritidis*] modification) were unrestricted by the corresponding hybrids which also possessed F101-102 (e.g., 4030 F′ 101-102). These other *Salmonella hsd* genes therefore differed from *hsdSB*, *hsdSP*, and *hsdSQ* (4, 6, 7).

Complementation of *Salmonella typhimurium* SB restriction function. When FS680-1 was transferred to the *E. coli*/*Salmonella* hybrid 4002, which has the $r_{SP}^+ m_{SP}^+$ genes from *S. potsdam*, SP-modified lambda phage was restricted. Thus r_{SB}^- was complemented by r_{SP}^+ as expected (4).

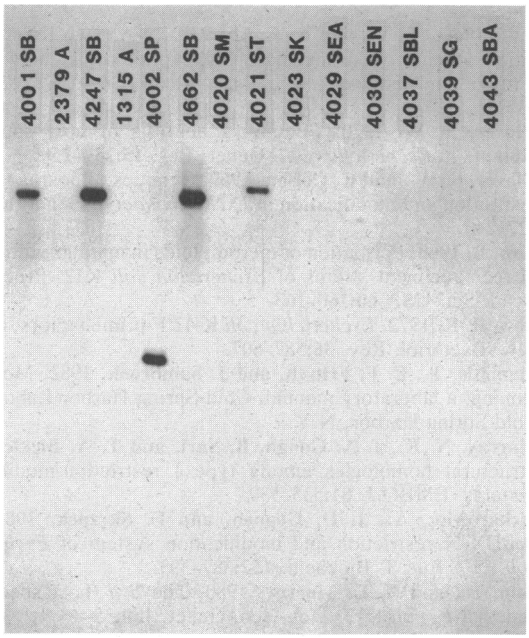

FIG. 1. Hybridization of *Hin*dIII-digested DNAs of *E. coli*, *S. typhimurium*, and *E. coli*/*Salmonella* hybrids to the 2.6-kilobase *Hin*dIII-*Eco*RI SB-specific probe. The source of the bacterial DNA is indicated at the top. DNA hybridizations were done by digesting bacterial DNAs with *Hin*dIII, separating the fragments in 1% agarose gels, and transferring them to Nytran membranes (Schleicher & Schuell, Inc.).

On the other hand, SM (*S. muenchen*)- and ST (*S. thompson*)-modified lambda phages plated out on the corresponding hybrids which possessed FS680-1 at EOPs between 0.1 and 0.5 (0.2 for λ SM and 0.3 for λ ST). It was possible, therefore, that r_{SB}^- was complemented by r_{SM}^+ and r_{ST}^+ but only weakly. However, lambda phages with SK (*S. kaduna*), SEA (*S. eastbourne*), SEN, SBL, (*S. blegdam*), SG (*S. gelsenkirchen*), and SBA (*S. bareilly*) modifications were unrestricted by the corresponding hybrids with FS680-1. Thus r_{SB}^+ may have been weakly complemented by the *Salmonella hsd* genes with SM and ST specificities but was not complemented by genes with the other *Salmonella* specificities.

DNA hybridization. We examined for hybridization of two *hsd* probes to *Hin*dIII-restricted DNA from each hybrid. These probes were a 1.1-kilobase *Eco*RI-*Bam*HI segment of the *E. coli hsdA* specificity determinants cloned in pFFP20 and called A specific (8) and a 2.6-kilobase *Hin*dIII-*Eco*RI fragment of the *hsdSB* genes of *S. typhimurium* which included all of *hsdM* and part of *hsdS* and was called SB specific (12). The plasmids were digested with the appropriate restriction endonucleases; the *hsd* fragments were extracted from the agarose, purified, nick translated, and hybridized to *Hin*dIII-digested bacterial DNAs.

The A-specific probe hybridized only to DNA from *E. coli* 2379, which has the *hsdA* genes, and failed to hybridize to DNA from any of the hybrids.

Results of hybridization of the SB-specific probe are shown in Fig. 1. This probe hybridized to DNAs from *S. typhimurium* 4247 and to DNA from hybrids 4662 and 4001, all of which have the *hsdSB* genes, as well as to DNA from hybrid 4002, which has the *hsdSP* genes of *S. potsdam*; all hybridizations were anticipated.

In addition, this SB-specific probe also hybridized to the DNA of hybrid 4021, which has the *hsdST* genes of *S. thompson*. However, there was no hybridization to the DNA of hybrid 4020 with *hsdSM* or to the DNA of any of the other hybrids. Thus, by weak complementation of r_{SB}^- and by hybridization with the SB-specific probe, the *hsdST* system of genes would be an additional member of the *E. coli* family of type 1 *hsd* genes. However, since the *hsdSM* genes failed to hybridize to the SB-specific probe, it is probable that the intermediate level of EOP obtained with this hybrid in the tests for complementation of r_{SB}^- was related to adsorption factors.

TABLE 2. Summary of (i) complementation tests between strains of *E. coli* with different restrictions and *E. coli*/*Salmonella* hybrids with different *Salmonella* restrictions and r_K^- on F101-102 and r_{SB}^- on FS680-1 and (ii) DNA-DNA hybridization tests between these same bacteria and the A- and SB-specific probes

Strain or hybrid	R-M phenotype	Complementation with[a]:		DNA hybridization with probe[b]:	
		r_K^-	r_{SB}^-	A-specific	SB-specific
2000	$r_B^+ m_B^+$	+	ND	ND	ND
2379	$r_A^+ m_A^+$	ND	ND	++	−
4662	$r_{SB}^+ m_{SB}^+$	+	+	−	++
4002	$r_{SP}^+ m_{SP}^+$	+	+	−	++
4020	$r_{SM}^+ m_{SM}^+$	−	(+)	−	−
4021	$r_{ST}^+ m_{ST}^+$	−	(+)	−	+
4023	$r_{SK}^+ m_{SK}^+$	−	−	−	−
4029	$r_{SEA}^+ m_{SEA}^+$	−	−	−	−
4030	$r_{SEN}^+ m_{SEN}^+$	−	−	−	−
4037	$r_{SBL}^+ m_{SBL}^+$	−	−	−	−
4039	$r_{SG}^+ m_{SG}^+$	−	−	−	−
4043	$r_{SBA}^+ m_{SBA}^+$	−	−	−	−

[a] ND, Not done; +, complementation (EOP of indicator phage lambda of 10^{-1} or less relative to the EOP on *E. coli* C); (+), possible complementation (EOP between 0.1 and 0.5); −, no complementation (EOP between 0.5 and 1.0). The EOPs of phage lambda with each of the *Salmonella* modifications on *E. coli* 1225 (r_K^+) and hybrid 4662 (r_{SB}^+) were all between 10^{-2} and 10^{-4}.

[b] ND, Not done; + and ++, densities of the bands on the autoradiograms, which are therefore visual estimates of the degrees of hybridization; −, no hybridization.

All results of the complementation and hybridization experiments are summarized in Table 2. From these results, it is clear that at least six *Salmonella hsd* genes neither complement r_K^- or r_{SB}^- nor hybridize with the A- or SB-specific probe. On the bases of these two criteria, these genes would be members of an additional family or families of the type I *hsd* genes.

Additional criteria might be required to define more clearly the status of the family. For example, it has been determined that there are 9 nucleotides between the methylated bases in the bipartite recognition sequence for *EcoA* but there are only 8 nucleotides separating the corresponding methylated nucleotides for all six members of the K family (22). This observation might be the result of a basic difference between different type I restriction endonucleases, sufficiently significant to place them into different families. Differences in the distances between the methylated bases in the recognition sites could also help explain the inability of members of the *E. coli* K family to complement *E. coli* A. Additional cloning and hybridization experiments are presently in progress.

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