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Two-Step Cloning and Expression in *Escherichia coli* of the DNA Restriction-Modification System *Sty*LTI of *Salmonella typhimurium*

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The *Sty*LTI restriction-modification system is common to most strains of the genus *Salmonella*, including *Salmonella typhimurium*. We report here the two-step cloning of the genes controlling the *Sty*LTI system. The *Sty*LTI methylase gene (*mod*) was cloned first. Then, the companion endonuclease gene (*res*) was introduced on a compatible vector. A strain of *S. typhimurium* sensitive to the coliphage lambda was constructed and used to select self-modifying recombinant phages from a Res$^-$ Mod$^+$ *S. typhimurium* genomic library in the λEMBL4 cloning vector. The methylase gene of one of these phages was then subcloned in pBR328 and transferred into *Escherichia coli*. In the second step, the closely linked endonuclease and methylase genes were cloned together on a single DNA fragment inserted in pACYC184 and introduced into the Mod$^+$ *E. coli* strain obtained in the first step. Attempts to transform Mod$^-$ *E. coli* or *S. typhimurium* strains with this Res$^+$ Mod$^+$ plasmid were unsuccessful, whereas transformation of Mod$^+$ strains occurred at a normal frequency. This can be understood if the introduction of the *Sty*LTI genes into naive hosts is lethal because of degradation of host DNA by restriction activity; in contrast to most restriction-modification systems, *Sty*LTI could not be transferred into naive hosts without killing them. In addition, it was found that strains containing only the *res* gene are viable and lack restriction activity in the absence of the companion *mod* gene. This suggests that expression of the *Sty*LTI endonuclease activity requires at least one polypeptide involved in the methylation activity, as is the case for types I and III restriction-modification systems but not for type II systems.

Restriction-modification systems (R-M systems) are biological systems that enable bacterial cells to make a distinction between their own DNA and foreign invasive DNA (phage DNA, for example). R-M systems consist of two highly specific enzymatic activities: an endonuclease and a DNA methylase which recognize the same specific sequence on DNA. After recognizing this sequence, the endonuclease cleaves both strands of the DNA within or outside the sequence. This function is called restriction. The methylase protects the resident DNA from restriction by methylating it at the specific sequence. This function is called modification. R-M systems have been classified into three groups, called types I, II, and III according to the complexity of their enzyme structures and their cofactor requirements. The three groups are also characterized by the structure of the DNA sequence recognized by the enzymes (see recent reviews in references 3, 35, 40, and 41). Mutations in the restriction-modification loci can result in two distinct phenotypes: either the loss of both restriction and modification functions (Res$^-$ Mod$^-$ phenotype) or the loss of the restriction function only (Res$^-$ Mod$^+$ phenotype).

There are three known R-M systems in *Salmonella typhimurium* LT2 and LT7: the *Sty*LTI, *Sty*SA, and *Sty*SB systems (14, 15, 18). All of them are encoded by chromosomal genes.

The *Sty*SB locus maps between *serB* and *pyrB* at 98 min on the chromosomal map of *S. typhimurium* (18). This type I system belongs to the Ia family proposed by Bickle (3). *Sty*SB genes are allelic to genes of the other members of the Ia family, including the well-characterized *Eco*K and *Eco*B systems, and to the *serB*-linked systems of other *Salmonella* strains (9, 39).

The *Sty*SA locus maps very close to the *Sty*SB locus. The molecular nature of *Sty*SA has not yet been investigated, so this system cannot yet be assigned to one of the three types of R-M systems. Nevertheless, recent work on Dar antirestriction activities of bacteriophage P1 suggests that *Sty*SA belongs to the Ib family, which includes the *Eco*A and *Eco*E systems (27).

Unlike the *serB*-linked systems (*Sty*SA and *Sty*SB), *Sty*LTI is common to most strains of the genus *Salmonella*, with the notable exceptions of *S. typhi*, *S. gallinarum*, and *S. pullorum* (10). In addition, and again in contrast with *Sty*SA and *Sty*SB, *Sty*LTI has the same DNA specificity in all *Salmonella* strains in which it is present. From an evolutionary point of view, it is surprising that *Sty*LTI remains invariable while the *serB*-linked systems show great plasticity for changes in specificity. On the basis of this observation, Bullas and coworkers (10) suggested that *Sty*LTI may possess a mechanism which prevents specificity changes, whereas such a mechanism would be missing in the *serB*-linked systems.

The *Sty*LTI locus maps close to *proC* at 8.5 min on the chromosomal map of *S. typhimurium* (16). A surprising result was obtained in the course of conjugative mating experiments when Pro$^+$ Res$^+$ Mod$^+$ Hfr strains were crossed with *proC* Res$^-$ Mod$^-$ recipient cells. Although a large number of Res$^+$ Mod$^+$ recombinants was expected among the selected Pro$^+$ recombinants, all of them conserved the Res$^-$ Mod$^-$ recipient phenotype (16). Similar results were obtained in transduction experiments between Res$^+$ Mod$^+$ and Res$^-$ Mod$^-$ strains (20a, 20d). This absence of recombinants expressing the donor Res$^+$ Mod$^+$ phenotype in crosses between wild-type donors and Res$^-$ Mod$^-$ recipient cells has never been observed in similar crosses involving other R-M systems (7, 12, 17, 18).

This paper suggests an explanation to this heretofore

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unexplained phenomenon. The genes coding for the *Sty*LTI modification methylase and restriction endonuclease were cloned sequentially. The modification-controlling gene was cloned first. Then, after modification of the host DNA had been accomplished, the restriction gene was introduced on a compatible vector. We show that the cloned *Sty*LTI genes are unable to become established efficiently in a Mod^- host, probably because of degradation of the host DNA by restriction activity. We show also that the *Sty*LTI restriction gene expresses Res^+ activity only when the gene is associated with the companion modification gene, suggesting that *Sty*LTI is a type I or a type III R-M system.

MATERIAL AND METHODS

Terminology. In accord with the recommendations of Smith and Nathans (36), the designation *Sty*LTI will be used to refer to the R-M system of *S. typhimurium* previously called LT (13). As recommended by Novick et al. (32), the abbreviations Res and Mod, possibly followed by the name of the relevant R-M system in parentheses, will be used to designate restriction and modification phenotypes or enzymatic activities. For the sake of clarity, the terms *res* and *mod* will refer to the genes responsible for *Sty*LTI restriction and modification activities, respectively, without implying that either one or more than one gene is responsible for the phenotypic trait.

Bacteria and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Both *Escherichia coli* and *S. typhimurium* strains were propagated in LB medium. For phage λ infection, *E. coli* was grown in LB plus 0.2% maltose. When necessary, antibiotics were added to the medium at the following final concentrations: sodium ampicillin, 50 μg/ml; chloramphenicol, 30 μg/ml; and tetracycline hydrochloride, 12.5 μg/ml. Bacteriophage λ stocks were prepared by the standard agar layer method in the presence of 10 mM $MgSO_4$.

DNA manipulations. Isolation of plasmid DNA, agarose gel electrophoresis of DNA, and transformation of *E. coli* were performed by standard techniques (30). Large-scale preparations of phage λ DNA were done as described by Dale and Greenaway (19, 20). Restriction endonucleases, calf intestine phosphatase, and T4 DNA ligase were obtained from Boehringer (Mannheim, Federal Republic of Germany) and used as recommended by the manufacturer. Recovery of DNA fragments from agarose gels was performed with a Geneclean kit obtained from BIO 101 (La Jolla, Calif.). A λ DNA in vitro packaging system was obtained from Amersham International (Little Chalfont, United Kingdom).

Measures of EOP of phages. An estimate of the efficiency of plating (EOP) of a phage was obtained by plating out 10-μl drops of suitable dilutions of the phage on the top layer of medium inoculated with the test strain of bacteria and comparing the numbers of plaques on this strain with the number of plaques obtained on a Res^- strain. For a more accurate determination of the EOP, 0.1-ml samples of serial dilutions of phage stock were allowed to adsorb at 37°C for 15 min in 0.25 ml of late-exponential-phase culture of bacteria before the phages were mixed with the top layer of the agar medium at 45°C and poured onto the surface of LB agar plates.

Growth of phage λ on *S. typhimurium*. Two obstacles prevent the growth of coliphage λ on *S. typhimurium*. First, λ does not adsorb to *S. typhimurium* because the organism lacks λ receptors (33). Second, the product of the *N* gene of λ is not functional in *S. typhimurium* because of the absence of an appropriate *nusA* gene in this species (21). The first problem can be overcome by introducing into *S. typhimurium* a multicopy plasmid (pAMH62) expressing *lamB*, the gene coding for the λ receptor of *E. coli* (24). The second problem can be circumvented by using N-independent (*nin*) derivatives of phage λ (23). In addition, we found that the *S. typhimurium* host strain must carry a *galE* mutation or other mutations leading to a rough phenotype to obtain visible plaques of λ (strain CL4701, Table 1).

Construction of a genomic library from a Res^- Mod^+ (*Sty*LTI) *S. typhimurium* strain in the λEMBL4 vector. High-molecular-weight chromosomal DNA of Res^- Mod^+ (*Sty* LTI) *S. typhimurium* CL4243 was purified by equilibrium centrifugation in a CsCl-ethidium bromide gradient (30). A sample of this DNA was subjected to *Sau*3AI partial digestion in order to obtain fragments of suitable sizes (10 to 20 kb) for subsequent ligation and packaging. The partially *Sau*3AI-digested DNA was dephosphorylated and mixed with λEMBL4 DNA previously cut with *Bam*HI and *Sal*I and precipitated with isopropanol (22). The mixture was ligated at a DNA concentration of 100 μg/ml and then packaged by using an in vitro packaging system. Samples of suitable dilutions were plated on both NM538 $(P2)^-$ and NM539 $(P2)^+$ in order to determine the proportion of recombinant phages by their Spi^- phenotype (22).

Detection of *Sty*LTI modification activity of recombinant plasmids by means of zygotic induction of phage P22 in *E. coli*. The Mod (*Sty*LTI) phenotype conferred by recombinant plasmids was determined by measuring the *Sty*LTI modification inherited by phage P22 after passage through *E. coli* strains containing the plasmid to be examined. Phage P22, whose natural host is *S. typhimurium*, does not adsorb to *E. coli*. Thus this phage was introduced into *E. coli* as a prophage carried by a conjugative F′ plasmid. The F′ *lac pro* (P22) contained in strain CL1250 was constructed as previously described (5). One milliliter of an overnight culture of CL1250 was mixed with 1 ml of an overnight culture of the strain to be examined in 10 ml of fresh LB medium. After 1 h of incubation at 37°C without agitation, the mixture was aerated until partial lysis occurred (in about 2 h). A sample of the lysate was sterilized with chloroform, and the EOP of the phage was measured on both Res^+ (*Sty*LTI) and Res^- (*Sty*LTI) *S. typhimurium* strains (CL4100 and CL4243, respectively).

Construction of a genomic library from a Res^+ Mod^+ (*Sty*LTI) *S. typhimurium* strain in the pACYC184 vector. High-molecular-weight chromosomal DNA of wild-type *S. typhimurium* CL4100 was purified and digested with *Sau*3AI as described above. The *Sau*3AI fragments were ligated with pACYC184 DNA linearized with *Bam*HI and dephosphorylated. The ligation mixture was transformed into Mod^+ (*Sty*LTI) CL1501. Transformed cells were selected on LB agar medium plates containing tetracycline and chloramphenicol.

Screening for Res^+ (*Sty*LTI) recombinant *E. coli* strains. Res^+ (*Sty*LTI) candidate clones were selected by their enhanced resistance to nonmodified λEMBL4 phage. Transformed colonies were replica plated on LB plates seeded with about 10^7 nonmodified particles of λEMBL4. The authentic Res^+ clones were identified among lysis-resistant colonies by their sensitivity to modified λEMBL4.

Curing of plasmids. In order to cure *E. coli* strains of plasmids, successive cultures of these strains were made in LB liquid medium in the absence of antibiotic selection. After several subcultures, antibiotic-sensitive colonies were isolated and screened for the absence of plasmid DNA.

TABLE 1. Bacterial strains and plasmids used in this study

Strains and plasmids	R-M phenotype	Relevant genotype or phenotype[a]	Source or reference
E. coli K-12			
HB101	Res$^-$ Mod$^-$ (*Eco*B)	*lacY1 galK2 rpsL20 xyl-5 mtl-1 recA13 ara-14 proA2 supE44 hsdSB20*	7
CL1250	Res$^-$ Mod$^-$ (*Eco*K)	*lacY1 tonA21 supE44 hsdSK918 leuB6 thi-1 thr-1 rpsL proC*::Tn*5*/F'*lac pro* (P22)	This study
CL1500	Res$^-$ Mod$^-$ (*Eco*B) Res$^-$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL510	This study
CL1501	Res$^-$ Mod$^-$ (*Eco*B) Res$^-$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL511	This study
CL1502	Res$^-$ Mod$^-$ (*Eco*B) Res$^+$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL511/pRUCL530	This study
CL1503	Res$^-$ Mod$^-$ (*Eco*B) Res$^+$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL530	This study
CL1504	Res$^-$ Mod$^-$ (*Eco*B) Res$^+$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL511/pRUCL531	This study
CL1505	Res$^-$ Mod$^-$ (*Eco*B) Res$^+$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL531	This study
CL1506	Res$^-$ Mod$^-$ (*Eco*B) Res$^+$ Mod$^+$ (*Sty*LTI)	HB101/pRUCL511/pRUCL532	This study
CL1507	Res$^-$ Mod$^-$ (*Eco*B) Res$^-$ Mod$^-$ (*Sty*LTI)	HB101/pRUCL532	This study
CL1510	Res$^-$ Mod$^-$ (*Eco*B)	HB101/pBR328	O. De Backer
NM538	Res$^-$ Mod$^+$ (*Eco*K)	*metB supF hsdR*	22
NM539	Res$^-$ Mod$^+$ (*Eco*K)	NM538 (P2 *cox3*)	22
S. typhimurium LT2			
CL4532	Res$^+$ Mod$^+$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	*metA metB trpB ilv-452 pyrB124 galE* P1^S	9
CL4701	Res$^+$ Mod$^+$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	CL4532/pAMH62, λ^S	This study
S. typhimurium LT7			
CL4100	Res$^+$ Mod$^+$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	Wild-type LT7	14
CL4243	Res$^-$ Mod$^+$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	*proC90 rpsL hsdLT123*	C. colson
CL4801	Res$^-$ Mod$^-$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	*proC90 hsdLT24 srl*::Tn*10 recA*	O. De Backer
CL4802	Res$^-$ Mod$^+$ (*Sty*LTI) Res$^+$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	*proC90 hsdLT243 srl*::Tn*10 recA*	O. De Backer
S. typhimurium-E. coli hybrid[b]			
CL4626	Res$^+$ Mod$^+$ (*Eco*K) Res$^-$ Mod$^+$ (*Sty*SA) Res$^+$ Mod$^+$ (*Sty*SB)	*leuB hsdRK* [*serB*$^+$ *hsdSA46 hsdSB6*]	C. Colson
Plasmids			
pBR328		Ap^r Cm^r Tc^r; 4.9 kb	37
pTZ18R		Ap^r *lacZ*, f1 *ori*, T7 promoter; 2.9 kb	Pharmacia
pACYC184		Cm^r Tc^r; 4.2 kb	11
pAMH62		*ompR-lamB* of *E. coli*, Ap^r	24
pRUCL510		[*Sty*LTI *mod*$^+$-*res23*] Ap^r Cm^s Tc^r; pBR328 replicon; 15.5 kb	This study
pRUCL511		[*Sty*LTI *mod*$^+$] Cm^s Tc^r; pBR328 replicon; 10.6 kb	This study
pRUCL520		[*Sty*LTI *mod*$^+$] Ap^r; pTZ18R replicon; 7.8 kb	This study
pRUCL521		[*Sty*LTI *mod*$^+$] Ap^r; pTZ18R replicon; 7.4 kb	This study
pRUCL530		[*Sty*LTI *mod*$^+$-*res*$^+$] Cm^r Tc^s; pACYC184 replicon; 17.2 kb	This study
pRUCL531		[*Sty*LTI *mod*$^+$-*res*$^+$] Cm^r Tc^s; pACYC184 replicon; 12.0 kb	This study
pRUCL532		[*Sty*LTI *res*$^+$ Δ*mod*] Cm^r Tc^s; pACYC184 replicon; 10.2 kb	This study

[a] Genes inside square brackets are from *S. typhimurium*.

[b] See reference 18 for the construction of similar hybrids which appear to have integrated a portion of the *S. typhimurium* chromosome including the *hsd*SA and *hsd*SB loci at a nonhomologous locus in the *E. coli* chromosome.

TABLE 2. EOP of phages

No.	Lysate: Phage[a]	EOP on strain: CL4701 Res$^+$ (*Sty*LTI)	EOP on strain: CL4626 Res$^-$ (*Sty*LTI)
1	λEMBL4. *Sty*SA, *Sty*SB, *Eco*K	1.7×10^{-7}	1.0
2	λEMBL4. *Sty*SA, *Sty*SB, *Sty*LTI	1.4	1.0
3[b]	Library. *Sty*SA, *Sty*SB, *Eco*K	1.9×10^{-4}	1.0
4	λMLTI1. *Sty*SA, *Sty*SB, *Eco*K	0.7	1.0
5	λMLTI1. *Sty*SA, *Sty*SB, *Sty*LTI	1.2	1.0

[a] Phage notations follow the system previously used in which the letters after the phage designation indicate the phage modification (2).

[b] Consisted of about 12,000 PFU of a genomic library of Res$^-$ Mod$^+$ (*Sty*LTI) *S. typhimurium* CL4243 amplified on CL4626.

RESULTS

Cloning of *Sty*LTI *mod* gene. Recombinant phages containing the gene for the *Sty*LTI modification enzyme were selected according to the method described by Borck et al. (4). This procedure is based on the assumption that recombinant phages containing the gene of a modification methylase can modify their own DNA when grown in a Mod$^-$ host. This allows one to perform an in vivo selection of self-modifying clones by exposing the library to the restriction exercised by a Res$^+$ strain.

In the case of *Sty*LTI, such a method for selecting the Mod$^+$ clones required two conditions to be fullfilled. First, the vector used had to be able to propagate on *S. typhimurium*. Second, it had to be sufficiently sensitive to *Sty*LTI restriction to allow effective selection of the clones.

The susceptibility of replacement vector λEMBL4 to *Sty*LTI restriction was measured on lambda-sensitive Res$^+$ (*Sty*LTI) *S. typhimurium* CL4701, obtained by introducing plasmid pAMH62 into the *galE* strain CL4532. Results given in Table 2 (lysates 1 and 2) show that λEMBL4 is strongly affected by the *Sty*LTI restriction: about one λEMBL4 nonmodified particle among 10^7 particles escaped the *Sty*LTI restriction activity exercised by CL4701. Thus a powerful selection could be exercised to select Mod$^+$ (*Sty*LTI) recombinant phages if they could ensure their own modification during the previous lytic cycle. A genomic library of the Res$^-$ Mod$^+$ (*Sty*LTI) *S. typhimurium* strain CL4243 was then constructed with the λEMBL4 vector. A sample of about 12,000 PFU of the library was amplified on CL4626 in order to allow host-induced modification of the library for systems *Sty*SA and *Sty*SB and self-modification of the desired clones for *Sty*LTI. Results in Table 2 show that the amplified library (lysate 3) plated on Res$^+$ (*Sty*LTI) CL4701 with an EOP about 1,000 times higher than that of the vector.

The self-modifying capacities of 12 recombinant phages randomly selected on CL4701 were examined. These 12 candidates were grown on Mod$^-$ CL4626 in order to eliminate the *Sty*LTI modification inherited from the selecting CL4701 while allowing them to perform their own modification. All 12 lysates thus obtained plated on CL4701 with an EOP of about 1, showing that they had most likely ensured their own *Sty*LTI modification and thus should contain and express the *Sty*LTI *mod* gene. One of these self-modifying phages, λMLTI1 (Table 2, lysates 4 and 5), was retained for further study.

A 10.6-kb *Eco*RI fragment of λMLTI1 containing the *mod* gene was subcloned in the *Eco*RI site of pBR328, and a restriction map was established (Fig. 1, pRUCL510). Selected fragments were then further subcloned into pBR328 and pTZ18R to establish whether these fragments were sufficient to confer the Mod$^+$ (*Sty*LTI) phenotype on HB101 (Fig. 1, pRUCL511, pRUCL520, and pRUCL521). The smallest fragment conferring modification activity was found to be the 4.5-kb fragment flanked by *Pst*I and *Sal*I sites in pRUCL 521.

Cloning of *Sty*LTI *res* gene. Since the *Sty*LTI *mod* gene had been subcloned into pMB1-derived plasmids, the compatible plasmid vector pACYC184 was used for cloning the companion *res* gene. A genomic library of Res$^+$ Mod$^+$ (*Sty*LTI) *S. typhimurium* CL4100 was constructed by using pACYC184 as vector and transformed into the Mod$^+$ (*Sty*LTI) *E. coli* CL1501 host strain. Res$^+$ clones were identified by

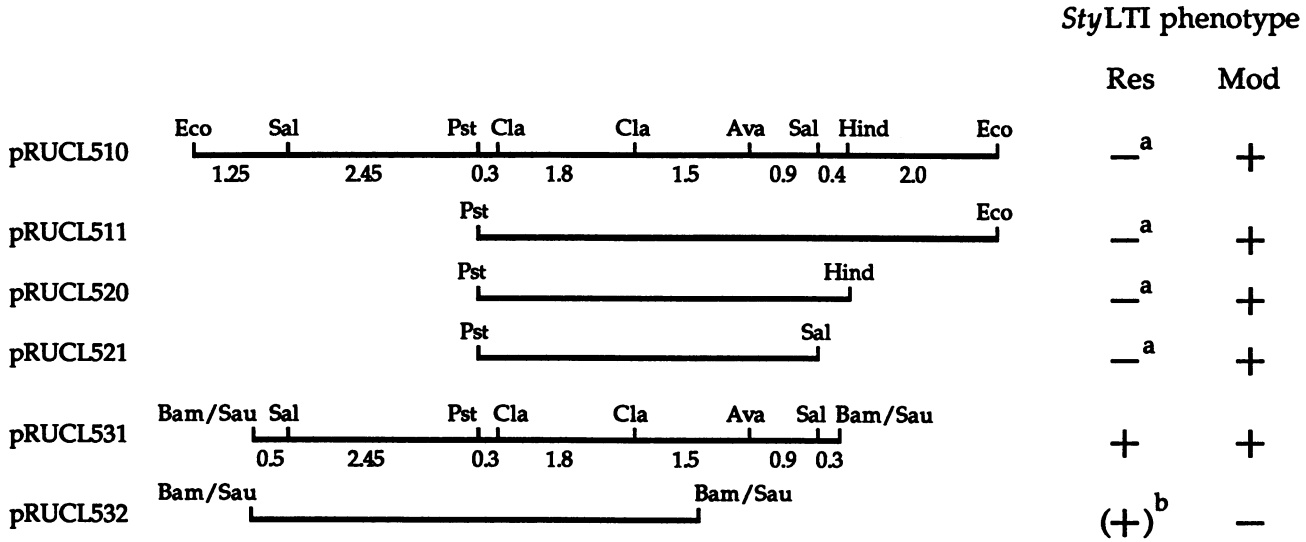

FIG. 1. Maps of the inserts of *Sty*LTI plasmids. Distances between restriction sites are given in kilobases. Restriction endonuclease sites: Ava, *Ava*I; Bam, *Bam*HI; Cla, *Cla*I; Eco, *Eco*RI; Hind, *Hin*dIII; Pst, *Pst*I; Sal, *Sal*I; Sau, *Sau*3AI. *Bam*HI and *Nco*I endonucleases do not cleave in any of the fragments shown. a, Insert of these plasmids derives from Res$^-$ Mod$^+$ (*Sty*LTI) *S. typhimurium* CL4243; b, this plasmid confers the Res$^+$ (*Sty*LTI) phenotype only when the host strain is Mod$^+$ (*Sty*LTI).

their increased ability to survive nonmodified λEMBL4 infection. Among resistant colonies, one (CL1502) was shown to exercise Res^+ (*Sty*LTI) activity: it was still fully sensitive to *Sty*LTI-modified λEMBL4 but restricted nonmodified λEMBL4 (EOP = 1.4×10^{-5}). Electrophoresis of plasmid DNA from CL1502 showed that in addition to pRUCL511, it contained a second plasmid, pRUCL530, presumed to be responsible for the Res^+ (*Sty*LTI) phenotype. As *res* and *mod* *Sty*LTI genes are closely linked on the chromosome of *S. typhimurium* (16), it was possible that, in addition to the selected *res* gene, the neighboring *mod* gene was also present on pRUCL530. In order to determine this, segregants of CL1502 that had lost pRUCL511 were selected. After being cured, strain CL1503 was found to remain Res^+ Mod^+, indicating that pRUCL530 carried both the *Sty*LTI *res* and *mod* genes.

Subcloning of *Sty*LTI genes: Res^+ activity is not expressed in the absence of Mod^+ activity. Plasmid pRUCL530 was found to contain an insert about 13 kb long. Fragments 4 to 9 kb long resulting from a partial *Sau*3AI digestion of pRUCL530 DNA were inserted into the *Bam*HI site of pACYC184. After transformation of Mod^+ *E. coli* CL1501, Res^+ Mod^+ clones were screened as described above, and five of them were further examined. These five clones were cured of pRUCL511 in order to determine whether the pRUCL530-derived fragment they contained comprised both the *res* and *mod* *Sty*LTI genes or only *res*.

After curing, subclone CL1505 was found to retain the Res^+ Mod^+ phenotype, indicating that both *Sty*LTI *res* and *mod* genes were present on the pACYC184-derived plasmid it contained. This plasmid was called pRUCL531. Restriction analysis of pRUCL531 showed an insert 7.75 kb long totally included within the insert of the Mod^+ plasmid pRUCL510 (Fig. 1).

The four other subclones became Res^- Mod^- after pRUCL511 was cured. However, when the pACYC184-derived plasmids these subclones contained were transformed back into Mod^+ *E. coli* CL1501, the phenotype switched to Res^+ Mod^+. Thus these subclones must contain a functional *res* gene that expresses Res^+ activity only when the gene is associated with the companion *mod* gene. In other words, the *Sty*LTI *res* gene remains cryptic in the absence of Mod^+ activity. Comparison of the physical map of one of these res^+ fragments (in pRUCL532) with that of the res^+ mod^+ insert of pRUCL531 suggests that *res* should be on the left and *mod* should be on the right in Fig. 1.

***Sty*LTI system in unable to become established in a naive host.** The isolation of the *Sty*LTI genes on a plasmid made it possible to investigate whether the lack of recombinants expressing the donor Res^+ Mod^+ phenotype in crosses with Res^- Mod^- recipients also occurred upon plasmid transformation. DNA from plasmid pRUCL531 was used to transform the otherwise isogenic Mod^+ and Mod^- *E. coli* strains CL1501 and CL1510. Results in Table 3 show that the Mod^+ (*Sty*LTI) character of the host was essential for the establishment of pRUCL531. Although both hosts were about equally able to be transformed by the control pACYC184 DNA, pRUCL531 transformants were obtained only with the Mod^+ recipient. A similar observation was made with *S. typhimurium* recipient strains (Table 3). No transformants containing pRUCL531 were obtained with the Res^- Mod^- recipient CL4801, while transformants occurred with the Res^- Mod^+ recipient CL4802.

In addition, no transformants were obtained when the mod^+ plasmid pRUCL511 was used to transform *E. coli* CL1507, which contains only the *res* gene (Table 3). Thus it is not the introduction of the *res* gene but rather its activity, which in this case is activated by the incoming *mod* gene, which causes the transformation failure.

TABLE 3. Transformation efficiency of Mod^+ and Mod^- (*Sty*LTI) *E. coli* and *S. typhimurium* strains

Transformed strain	*Sty*LTI phenotype	No. of transformed colonies/μg of DNA from:		
		pACYC184	pRUCL531	pRUCL511
E. coli				
CL1510	Res^- Mod^-	4.9×10^5	0[a]	5.3×10^5
CL1501	Res^- Mod^+	2.7×10^5	1.3×10^3	ND
CL1507	Res^- Mod^-	ND[b]	ND	0[a]
S. typhimurium				
CL4801	Res^- Mod^-	1.2×10^5	0[a]	ND
CL4802	Res^- Mod^+	2.3×10^5	9.7×10^2	ND

[a] No transformed colonies were obtained with 10, 100, or 1,000 ng of DNA.
[b] ND, Not determined.

DISCUSSION

Earlier observations (16, 20a, 20d) concerning the apparent loss of linkage between *Sty*LTI genes and *proC* in conjugative crosses and transductions between Res^+ Mod^+ donors and Res^- Mod^- recipients were very unusual. Theoretically, there were three possible ways to explain these unexpected observations. First, the loss might be due to some irregularities at the level of recombination between the transferred and resident *Sty*LTI loci. Second, it might be due to a lack of expression of the wild-type *Sty*LTI genes established in a Mod^- host. Third, it might be related to the special situation of zygotes when the resident DNA is not modified but the zygotes have inherited a set of genes governing the ability to degrade unmodified DNA. Such a situation could be lethal to the zygotes. However, no such zygotic lethality was observed upon genetic analysis of other R-M systems (7, 12, 17, 18).

The cloning of the *Sty*LTI genes permitted an examination of the transfer and expression of these genes into Mod^- (*Sty*LTI) hosts without involvement of the recombination process. In order to ensure the establishment and expression of the *Sty*LTI genes, these genes were cloned sequentially. The *mod* gene was cloned first, and then, after modification of the host cells had taken place, the *res* gene was introduced. The establishment of the cloned *Sty*LTI system into a new host was shown to be strictly dependent on the previous *Sty*LTI modification ability of the host. This observation suggests that the introduction of *Sty*LTI genes in naive hosts is lethal because of degradation of host DNA by the restriction activity. Work reported separately (20b) confirms this hypothesis by showing that the transfer of a conjugative plasmid carrying the *Sty*LTI genes induces the death of nonmodified recipient cells as the consequence of the digestion of their genomic DNA. In contrast to most R-M systems, *Sty*LTI could not be transferred into naive hosts without killing them. Thus the two-step cloning strategy was wise. Any attempt to clone both the *Sty*LTI *res* and *mod* genes by a single-step method would have failed.

Two other systems, *Dde*I (25) and *Bam*HI (8), appear to have the same killing behavior. The cloning into *E. coli* of the genes controlling these systems required the introduction and expression of the methylase gene before the endonuclease gene could be introduced. However, the situations with

*Dde*I and *Bam*HI may not be directly comparable to that of *Sty*LTI. The distant phylogenetic origins of *Dde*I and *Bam*HI could explain their inabilities to regulate the expression of the endonuclease gene after transfer of the gene into *E. coli*. Very little is known about the mechanisms allowing the establishment of R-M systems in new nonmodified bacterial hosts. Putative regulatory mechanisms have been proposed, especially for *Eco*RV (6), *Pae*R7 (38), *Taq*I (34), and *Fok*I (28). On the other hand, engineered *E. coli* cells carrying and expressing some type II endonuclease genes are viable, although poorly, even when they are unmodified (see reference 29 for a review). This viability of some Res^+ Mod^- constructs suggests the possibility that *E. coli* can repair the endonucleolytic damages that occur during the establishment of new R-M systems.

The lethal character of the inheritance of the *Sty*LTI genes by a nonmodified host could also provide an explanation of the invariable specificity of the *Sty*LTI system in *Salmonella* species (10). Indeed, if a mutation in the *Sty*LTI locus were to lead to a change of specificity, the host cell would be in a situation comparable to that of a nonmodified host having inherited the *Sty*LTI system.

The isolation of DNA fragments having no detectable Res or Mod activity in a Mod^- host cell but leading to the Res^+ phenotype when introduced into a Mod^+ host suggests that *Sty*LTI is not a type II system. Indeed, the activity of a type II endonuclease is independent of the presence of the companion methylase. Conversely, type I and III restriction polypeptides (coded by the genes *hsdR* and *res*, respectively) have no activities by their own. They are subunits of multifunctional enzymes mediating both Res and Mod activities.

We have found that the structure of the nucleotide sequence recognized by the *Sty*LTI enzymes is very similar to that of the three known type III enzymes, *Eco*P1, *Eco*P15 and *Hin*fIII (20c). This suggests that *Sty*LTI could be a fourth type III R-M system. Systems *Eco*P1 and *Eco*P15, coded respectively by temperate phage P1 and plasmid p15B, have been cloned without difficulty in a single step into naive *E. coli* host strains (26, 31). The mechanism permitting this establishment is still unknown. However, it has been shown that upon phage P1 infection and lysogenization, *Eco*P1 modification is expressed much faster than restriction (1), allowing full protection of the resident DNA and establishment of the P1 prophage in its new host. R-M systems encoded by plasmids and phages must be designed for horizontal transfer into new hosts. In contrast, the inability of the *Sty*LTI system to be transferred into nonmodified hosts could be related to the fact that it is located on the chromosome.

In addition to a defensive action against infection by phages, R-M systems also limit exchanges of genetic information between bacteria and may constitute an important factor in the speciation phenomenon. The presence of the invariant *Sty*LTI system in most serotypes of *Salmonella* species (10) suggests that this system could have played an important part in the evolution of the salmonellae. Indeed, the emergence in an ancestral bacterial cell of an R-M system unable to be transferred to other cells or to change specificity could have led to strong genetic isolation of its progeny.

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REFERENCES

1. **Arber, W., and D. Dussoix.** 1962. Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ. J. Mol. Biol. **5**:18–36.
2. **Arber, W. A., and S. Linn.** 1969. DNA modification and restriction. Annu. Rev. Biochem. **38**:467–500.
3. **Bickle, T. A.** 1987. DNA restriction and modification systems, p. 692–696. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
4. **Borck, K., J. D. Beggs, W. J. Brammar, A. S. Hopkins, and N. E. Murray.** 1976. The construction *in vitro* of transducing derivatives of phage lambda. Mol. Gen. Genet. **146**:199–207.
5. **Botstein, D., and I. Herskowitz.** 1974. Properties of hybrids between *Salmonella* phage P22 and coliphage λ. Nature (London) **251**:584–589.
6. **Bougueleret, L., M. Schwarzstein, A. Tsugita, and M. Zabeau.** 1984. Characterization of the genes coding for the *Eco*RV restriction and modification system of *Escherichia coli*. Nucleic Acids Res. **12**:3659–3676.
7. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–479.
8. **Brooks, J. E., J. S. Benner, K. R. Silber, D. F. Heiter, L. A. Sznyter, T. Jager-Quinton, G. G. Wilson, L. S. Moran, B. E. Slatko, and D. O. Nwankwo.** 1988. Cloning and characterization of the *Bam*HI restriction modification system. Gene **74**:13.
9. **Bullas, L. R., and C. Colson.** 1975. DNA restriction and modification systems in *Salmonella*. III. SP, a *Salmonella postdam* system allelic to the SB system in *Salmonella typhimurium*. Mol. Gen. Genet. **139**:177–188.
10. **Bullas, L. R., C. Colson, and B. Neufeld.** 1980. Deoxyribonucleic acid restriction and modification systems in *Salmonella*: chromosomaly located systems of different serotypes. J. Bacteriol. **141**:275–292.
11. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
12. **Colson, A. M., and C. Colson.** 1972. Expression of the *Escherichia coli* K, B and phage P1 DNA host specificities in *Salmonella typhimurium*. J. Gen. Microbiol. **70**:123–128.
13. **Colson, A. M., C. Colson, and A. Van Pel.** 1969. Host-controlled restriction mutants of *Salmonella typhimurium*. J. Gen. Microbiol. **58**:57–64.
14. **Colson, C., and A. M. Colson.** 1967. Host specificity and fertility in *Salmonella typhimurium* LT7. Biochem. Biophys. Res. Commun. **29**:692–695.
15. **Colson, C., and A. M. Colson.** 1971. A new *Salmonella typhimurium* DNA host specificity. J. Gen. Microbiol. **69**:345–351.
16. **Colson, C., A. M. Colson, and A. Van Pel.** 1970. Chromosomal location of host specificity in *Salmonella typhimurium*. J. Gen. Microbiol. **60**:265–271.
17. **Colson, C., S. W. Glover, N. D. Symonds, and K. A. Stacey.** 1965. The location of the genes for host controlled modification and restriction in *Escherichia coli* K12. Genetics **52**:1043–1050.
18. **Colson, C., and A. Van Pel.** 1974. DNA restriction and modification systems in *Salmonella*. I. SA and SB, two *Salmonella typhimurium* systems determined by genes with a chromosomal location comparable to that of the *Escherichia coli hsd* genes. Mol. Gen. Genet. **129**:325–337.
19. **Dale, J. W., and P. J. Greenaway.** 1984. Preparation and assay of phage lambda, p. 201–209. *In* J. M. Walker (ed.), Methods in molecular biology, vol. 2. Nucleic acids. Humana Press, Clifton, N.J.
20. **Dale, J. W., and P. J. Greenaway.** 1984. Preparation of phage lambda DNA, p. 211–215. *In* J. M. Walker (ed.), Methods in molecular biology, vol. 2. Nucleic acids. Humana Press, Clif-

ton, N.J.
20a. **De Backer, O., and C. Colson.** Unpublished data.
20b. **De Backer, O., and C. Colson.** 1991. Transfer of the genes for the *Sty*LTI restriction-modification system of *Salmonella typhimurium* to strains lacking modification ability results in death of the recipient cells and degradation of their DNA. J. Bacteriol. **173**:1328–1330.
20c. **De Backer, O., and C. Colson.** Gene, in press.
20d. **Digneffe, C.** 1977. Ph.D. thesis. Université Catholique de Louvain, Louvain-la-Neuve, Belgium.
21. **Friedman, D. I., and E. R. Olson.** 1983. Evidence that a nucleotide sequence, "*boxA*," is involved in the action of the NusA protein. Cell **34**:143–149.
22. **Frischauf, A.-M., H. Lehrach, A. Poutska, and N. Murray.** 1983. Lambda replacing vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
23. **Harkki, A., and E. T. Palva.** 1984. Application of phage lambda technology to *Salmonella typhimurium*. Construction of a lambda-sensitive *Salmonella* strain. Mol. Gen. Genet. **195**:256–259.
24. **Harkki, A., and E. T. Palva.** 1985. A *lamB* expression plasmid for extending the host range of phage λ to other enterobacteria. FEMS Microbiol. Lett. **27**:183–187.
25. **Howard, K. A., C. Card, J. S. Benner, H. L. Callahan, R. Maunus, K. Silber, G. Wilson, and J. E. Brooks.** 1986. Cloning the *Dde*I restriction-modification system using a two-step method. Nucleic Acids Res. **17**:7939–7951.
26. **Iida, S., J. Meyer, B. Bächi, M. Stalhammar-Carlemalm, S. Schrickel, T. A. Bickle, and W. Arber.** 1983. DNA restriction-modification genes of phage P1 and plasmid p15B. J. Mol. Biol. **165**:1–18.
27. **Iida, S., M. B. Streiff, T. A. Bickle, and W. Arber.** 1987. Two DNA antirestriction systems of bacteriophage P1, darA, and darB: characterisation of darA$^-$ phages. Virology **157**:156–166.
28. **Looney, M. C., L. S. Moran, W. E. Jack, G. R. Feehery, J. S. Benner, B. E. Slatko, and G. G. Wilson.** 1989. Nucleotide sequence of the *Fok*I restriction-modification system: separate strand-specificity domains in the methyltransferase. Gene **80**: 193–208.
29. **Lunnen, K. D., J. M. Barsomian, R. R. Camp, C. O. Card, S.-Z. Chen, R. Croft, M. C. Looney, M. M. Meda, L. S. Moran, D. O. Nwankwo, B. E. Slatko, E. M. Van Cott, and G. G. Wilson.** 1988. Cloning type-II restriction and modification genes. Gene **74**:25–32.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Mural, R. J., R. H. Chesney, and D. Vapnek.** 1979. Isolation and characterization of cloned fragments of bacteriophage P1 DNA. Virology **93**:387–397.
32. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. C. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
33. **Schwartz, M., and L. Le Minor.** 1975. Occurrence of the bacteriophage lambda receptor in some *Enterobacteriaceae*. J. Virol. **15**:679–685.
34. **Slatko, B. E., J. S. Benner, T. Jager-Quinton, L. S. Moran, T. G. Simcox, E. M. Van Cott, and G. G. Wilson.** 1987. Cloning, sequencing and expression of the *Taq*I restriction-modification system. Nucleic Acid Res. **15**:9781–9796.
35. **Smith, H. O., and S. V. Kelly.** 1984. Methylases of the type II restriction-modification systems, p. 39–71. *In* A. Razin, H. Cedar, and A. D. Riggs (ed.), DNA methylation: biochemistry and biological significance. Springer-Verlag, New York.
36. **Smith, H. O., and D. Nathans.** 1973. A suggested nomenclature for bacterial host modification and restriction systems and their enzymes. J. Mol. Biol. **81**:419–423.
37. **Soberon, X., L. Covarrubias, and F. Bolivar.** 1980. Construction and characterisation of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. Gene **9**:287–305.
38. **Theriault, G., P. H. Roy, K. A. Howard, J. S. Benner, J. E. Brooks, A. F. Waters, and T. R. Gingeras.** 1985. Nucleotide sequence of the *Pae*R7 restriction/modification system and partial characterization of its protein products. Nucleic Acid Res. **13**:8441–8460.
39. **Van Pel, A., and C. Colson.** 1974. DNA restriction and modification systems in *Salmonella*. II. Genetic complementation between the K and B systems of *Escherichia coli* and the *Salmonella typhimurium* system SB, with the same chromosomal location. Mol. Gen. Genet. **135**:51–60.
40. **Wilson, G. G.** 1988. Type II restriction-modification systems. Trends Genet. **4**:314–318.
41. **Yuan, R., and L. Hamilton.** 1984. Type I and type III restriction-modification enzymes, p. 11–37. *In* A. Razin, H. Cedar, and A. D. Riggs (ed.), DNA methylation: biochemistry and biological significance. Springer-Verlag, New York.