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NOTES

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

OLIVIER DE BACKER AND CHARLES COLSON*

Unité de Génétique, Département de Biologie, Université Catholique de Louvain, Place Croix du Sud 4, B-1348 Louvain-la-Neuve, Belgium

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The genes encoding the restriction-modification system *Sty*LTI of *Salmonella typhimurium* were inserted in vivo into the conjugative plasmid pULB21. This allowed us to transfer the *Sty*LTI genes at a very high frequency and to monitor the fate of recipient cells after mating. Transfer of the *Sty*LTI restriction and modification genes into a modificationless recipient was lethal and resulted in degradation of the cell's DNA. This indicates that, in contrast to any other known restriction-modification systems, *Sty*LTI cannot be established after horizontal transfer into a naive host.

The *Sty*LTI restriction-modification system (R-M system) is present in most strains of the genus *Salmonella*, including the well-characterized strains *S. typhimurium* LT2 and LT7, where it was first detected (3, 4).

Several observations suggest that *Sty*LTI could belong to the type III family of R-M systems, which includes *Eco*P1, *Eco*P15, and *Hin*fIII (for a review, see reference 2). First, the *Sty*LTI recognition sequence 5′-CAGAG-3′ has an asymmetric and nonhyphenated structure similar to that of the three known type III enzymes (6). Second, the *Sty*LTI methylase transfers a single methyl group at each unmodified recognition site (on the 3′A of the sequence). Thus, only one of the two DNA strands of a modified *Sty*LTI site is methylated. Third, it was found that *Sty*LTI restriction and modification activities are governed by at least two genes, which were called *res* and *mod* by analogy to the genes encoding the type III enzymes. The *res* gene is necessary only for restriction activity, while *mod* is required for both restriction and modification activities.

In a separate paper (7), we report the two-step cloning in *Escherichia coli* of the *Sty*LTI genes. The methylase gene (*mod*) was cloned first, and then, after methylation of the host DNA had taken place, the endonuclease genes (*res* and *mod*) were introduced on a compatible plasmid. The ability to transform new bacterial hosts with a plasmid containing *Sty*LTI *res* and *mod* genes was shown to be strictly dependent on the previous modification ability of the host. This suggested that the transfer of *Sty*LTI into naive cells is lethal because of digestion of nonmodified host DNA by the endonuclease. The work reported here confirms this hypothesis by showing that conjugative transfer at a very high frequency of a plasmid carrying the *Sty*LTI genes to a nonmodified host results in death of the recipient cells and degradation of their DNA.

In order to examine the fate of cells having just inherited the *Sty*LTI genes, we constructed conjugative plasmids able to transfer these genes at high frequency into new bacterial hosts. These plasmids were obtained by cointegration between pULB21, which is a transposable conjugative plasmid, and pRUCL531, which is a derivative of plasmid pACYC184 containing both the *Sty*LTI *res* and *mod* genes. Plasmid pULB21 is a derivative of the broad-host-range conjugative plasmid RP4 (Kn^r Ap^r Tc^s) obtained by insertion of mini-Mu transposon Mu3A into the tetracycline resistance gene of RP4 (10). Mu3A can promote the random integration of pULB21 into almost any DNA molecule, leading to the fusion of the two replicons (for reviews, see references 5 and 9).

Plasmid pULB21 (Kn^r Ap^r) was transferred into *E. coli* CL1505, a derivative of HB101 containing pRUCL531 [Res^+ Mod^+ (*Sty*LTI) Cm^r]. This strain, now containing pULB21 and pRUCL531, was mated with CL1501, which is a derivative of HB101 expressing the *Sty*LTI methylase gene carried on plasmid pRUCL511 [Mod^+ (*Sty*LTI) Tc^r]. Kn^r Cm^r Tc^r transconjugants thought to contain pULB21-pRUCL531 cointegrates were selected. Because Mu3A integrates at essentially random sites in the target DNA molecule, fusion between pULB21 and pRUCL531 could disrupt any genes of pRUCL531, including the *Sty*LTI genes. Thus, three possible kinds of cointegrates were expected, depending on the location of the fusion point: outside the *Sty*LTI genes, into the *mod* gene, or into the *res* gene. Indeed, three classes of Kn^r Cm^r Tc^r transconjugants were obtained.

Transconjugants of the first class were restriction and modification proficient (Res^+ Mod^+) as judged by their abilities to restrict and modify λ phages. After curing of pRUCL511, these transconjugants remained Res^+ Mod^+, indicating that they contained cointegrates with functional *res* and *mod* genes. In addition, when they were mated with the Mod^+ *E. coli* strain CL1801 (*recA56* Rif^r, pRUCL511), transfer of Kn^r was shown to be strictly associated with transfer of the Res^+ phenotype. Thus, this first type of

* Corresponding author.

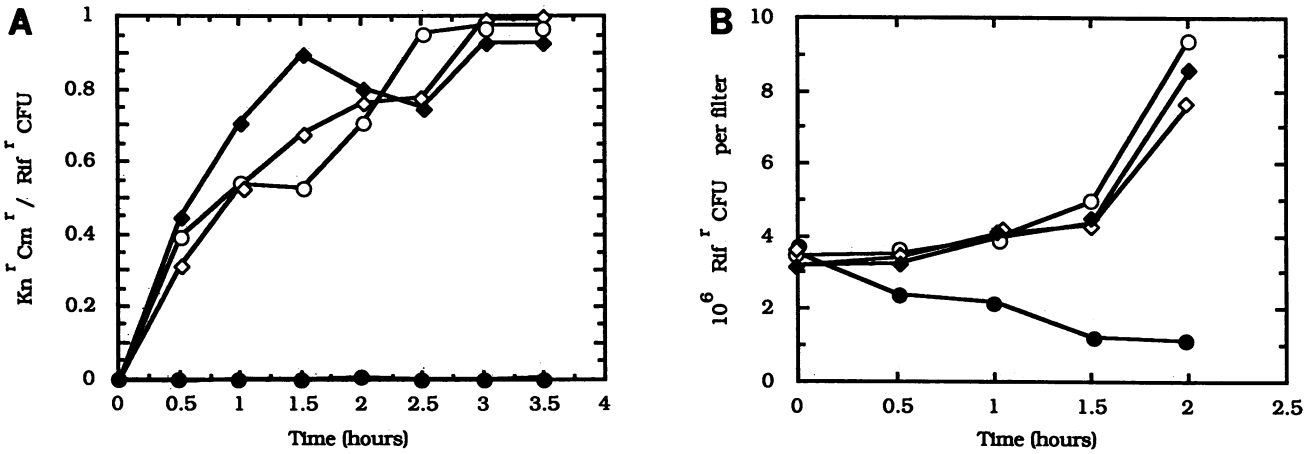

FIG. 1. Time course of conjugative transfer of *Sty*LTI genes to Mod$^+$ and Mod$^-$ *E. coli* strains. Strains CL1520 and CL1521, which contain, respectively, the Kn^r Cm^r conjugative plasmids pRUCL541 (Res$^+$ Mod$^+$) and pRUCL542 (Res$^-$ Mod$^+$), were mated with the Rif^r *E. coli* strains CL1800 (Mod$^-$) and CL1801 (Mod$^+$). Symbols: ●, CL1520 (Res$^+$ Mod$^+$) × CL1800 (Res$^-$ Mod$^-$); ○, CL1521 (Res$^-$ Mod$^+$) × CL1800 (Res$^-$ Mod$^-$); ◆, CL1520 (Res$^+$ Mod$^+$) × CL1801 (Res$^-$ Mod$^+$); ◇, CL1521 (Res$^-$ Mod$^+$) × CL1801 (Res$^-$ Mod$^+$). (A) Apparent efficiency of transfer. (B) Viability of recipient cells.

transconjugants must contain pULB21-pRUCL531 cointegrates in which fusion of the replicons occurred outside the *Sty*LTI genes.

Transconjugants of the second class were Res$^-$ Mod$^+$, remained Res$^-$ Mod$^+$ after curing of pRUCL511, and were able to transfer the Mod$^+$ phenotype when crossed with *E. coli* CL1800 (*recA56* Rif^r, pBR328). Thus, they contained pULB21-pRUCL531 cointegrates with a disrupted *res* gene.

Transconjugants of the third class were Res$^-$ Mod$^+$, like those of the second class, but they became Res$^-$ Mod$^-$ after curing of pRUCL511. One possibility is that they contained cointegrates in which the disruption of the *mod* gene had a polar effect on the expression of the *res* gene.

Some of the constructed cointegrates were used to examine the effect of the transfer of the *Sty*LTI genes into modified or nonmodified recipient cells.

Transconjugants CL1520 and CL1521, which contain, respectively, the cointegrates pRUCL541 (Res$^+$ Mod$^+$) and pRUCL542 (Res$^-$ Mod$^+$), were mated with the otherwise isogenic Mod$^-$ and Mod$^+$ *E. coli* strains CL1800(pBR328) and CL1801(pRUCL511). Donor and recipient cells were grown at 37°C in 2× YT medium (tryptone, 16 g/liter; yeast extract, 10 g/liter; NaCl, 5 g/liter; pH 7.0) supplemented with appropriate antibiotics until cell density reached about 2 × 10^8 cells per ml. Cells were then harvested by centrifugation, suspended in the same volume of prewarmed 2× YT medium without antibiotics, and incubated for 1 h at 37°C with gentle aeration. Donor and recipient cultures were then mixed at a 10:1 ratio. Mating was initiated by spotting 50-μl drops of the mixture on individual HAWP 0.45-μm-pore-size Millipore filters laid on prewarmed 2× YT plates. At intervals, filters were removed and the cells were eluted by vortexing them in 40 ml of saline buffer (NaCl, 0.85% [wt/vol], 0.066 M $NaPO_4$; pH 7.0) and then further diluted. Portions from the appropriate dilutions were spread on 2× YT plates containing rifampin (100 μg/ml), kanamycin (25 μg/ml), and chloramphenicol (15 μg/ml) to select for transconjugants which had inherited the cointegrate of the donor.

Results in Fig. 1A show that the Res$^+$ Mod$^+$ cointegrate pRUCL541 was almost never transferred from CL1520 to the Mod$^-$ recipient CL1800, while transfer was very efficient when the recipient was Mod$^+$ CL1801. In contrast, the Res$^-$ Mod$^+$ cointegrate pRUCL542 was transferred with the same high efficiency from CL1521 to both CL1800 and CL1801. This indicated that CL1800 is as good a recipient as CL1801 in conjugation. Thus, the lack of transfer of Res$^+$ Mod$^+$ pRUCL541 from CL1520 to CL1800 was a result of the Mod$^-$ character of CL1800 rather than the inability of strain CL1800 to act as a good recipient in conjugation. On the other hand, the Res$^-$ Mod$^+$ cointegrate pRUCL542 was transferred at high frequency from CL1521 to Mod$^-$ CL1800. This indicated that the inability of pRUCL542 to be transferred to CL1800 cannot be attributed to a possible deleterious effect of methylase activity in a nonmodified host.

Thus, these results confirm those obtained in transformation experiments (7) and suggest that the inability of *Sty*LTI to be established in naive hosts is caused by a premature expression of the endonuclease activity that leads to death of the recipient cell as a consequence of the degradation of its genomic DNA.

Figure 1B represents the evolution of the viability of recipient cells during the course of the four matings referred to in the legend to Fig. 1A. After 120 min of conjugation, the number of CFU of Mod$^-$ CL1800 dropped about fourfold when this strain was mated with CL1520 containing Res$^+$ Mod$^+$ pRUCL541. Meanwhile, the number of CFU increased about twofold when the donor was CL1521 with Res$^-$ Mod$^+$ pRUCL542. In contrast, the number of CFU of the Mod$^+$ recipient CL1801 evolved in similar ways when this strain was mated with CL1520 or CL1521. This indicated that it is the nonmodified character of the CL1800 strain that causes its death during mating with CL1520. Thus, transfer of the *Sty*LTI genes into a Mod$^-$ recipient cell is a lethal event.

A likely hypothesis is that this lethality is caused by restriction of the nonmodified recipient chromosomal DNA. To check this possibility, we have investigated the fate of ^{3}H-labeled DNA of Mod$^+$ and Mod$^-$ recipient cells during transfer of the *Sty*LTI genes. Results shown in Fig. 2 are a direct demonstration that transfer of the *Sty*LTI genes into a Mod$^-$ cell leads to degradation of the cell DNA. The Res$^+$ Mod$^+$ pRUCL541 and Res$^-$ Mod$^+$ pRUCL542 cointegrates were transferred into CL1802(pBR328) and CL1803

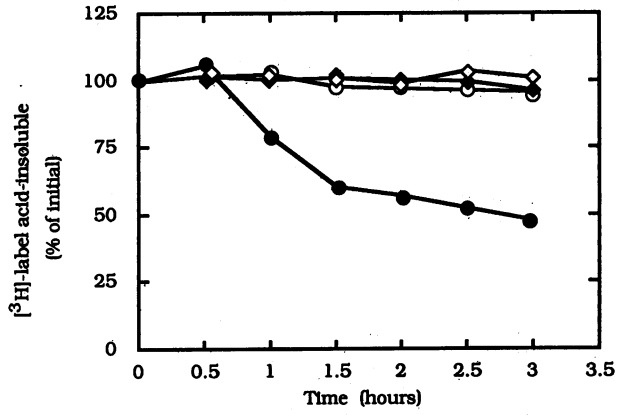

FIG. 2. DNA breakdown during transfer of *Sty*LTI genes into a Mod$^-$ recipient strain. The Mod$^-$ CL1802 and Mod$^+$ CL1803 recipient strains were labeled with [^{3}H]thymidine before being mated with CL1520 and CL1521, which contain, respectively, the conjugative plasmids pRUCL541 (Res$^+$ Mod$^+$) and pRUCL542 (Res$^-$ Mod$^+$). Symbols: ●, CL1520 (Res$^+$ Mod$^+$) × CL1802 (Res$^-$ Mod$^-$); ○, CL1521 (Res$^-$ Mod$^+$) × CL1802 (Res$^-$ Mod$^-$); ◆, CL1520 (Res$^+$ Mod$^+$) × CL1803 (Res$^-$ Mod$^+$); ◇, CL1521 (Res$^-$ Mod$^+$) × CL1803 (Res$^-$ Mod$^+$).

(pRUCL511), which are otherwise isogenic Mod$^-$ and Mod$^+$ derivatives of *E. coli* TG1 (*supE hsdΔ5 thi Δlac-proAB* F′ [*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]). Before strains were mated, DNA of the recipients was radioactively labeled in order to detect any subsequent degradation.

The recipient cells were grown at 37°C with aeration in M9 minimal medium supplemented with 0.1% (wt/vol) Casamino Acids. At a titer of about 2 × 10^8 cells per ml, 5 μCi of [*methyl*-^{3}H]thymidine per ml (specific activity, 45 Ci/mmol) was added for 15 min. The cells were harvested by centrifugation, washed three times in 2× YT medium containing nonradioactive thymidine (50 μg/ml), and then mixed with a 10-fold excess of donor cells. Matings were performed on filters as described above, except that nonradioactive thymidine (50 μg/ml) was added to the 2× YT plates. At intervals, cells were eluted in 20 ml of chilled saline buffer, and 5 ml of 30% trichloroacetic acid was added. The precipitates were collected on glass fiber filters (GFC Whatman), and radioactivity was determined by liquid scintillation counting. Results in Fig. 2 show that transfer of the *Sty*LTI genes from CL1520 to CL1802 led to a degradation of recipient DNA so that after 180 min of conjugation, more than 50% of the ^{3}H label became acid soluble. In contrast, DNA degradation was not observed with the Mod$^+$ recipient or when only the *mod* gene was transferred. This result implies that, after transfer of the genes into a new host, expression of the *Sty*LTI restriction activity is not delayed until complete protection of the host DNA is achieved by methylation activity. This is in contrast to most other R-M systems, which can be freely transferred to nonmodified cells (for example, see references 1 and 8). The nature of the control mechanisms allowing the horizontal transfer of R-M systems into new hosts remains to be elucidated.

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