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Delayed Expression of In Vivo Restriction Activity following Conjugal Transfer of *Escherichia coli* hsd_K (Restriction-Modification) Genes

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Following conjugal transfer of the hsd_K genes ($hsdR_K$, $hsdM_K$, and $hsdS_K$) of *Escherichia coli* K-12, restriction activity was first detected only after approximately 15 generations, whereas modification activity was observed immediately. This sequential expression explains the establishment of hsd_K genes in a nonmodified host and suggests regulation of restriction activity after conjugal transfer.

Type I restriction and modification (R-M) systems are the most complex known R-M systems because of their enzyme structure, mechanism of action, and requirements (13). Type I R-M genes can be transferred by conjugation, transfection, or transformation to other strains which lack the modification for the systems transferred (5, 11). Establishment of R-M genes in a nonmodified recipient could present a problem unless there is control of the restriction activity. As early as 1964, successful conjugation of wild-type hsd_K genes to a nonmodified recipient was demonstrated (2, 4). However, a detailed study of phenotypic expression of R-M genes immediately following conjugal transfer has not been reported. In this study, we transferred an F′ plasmid containing the complete hsd_K genes into a non-K-modified recipient and then examined the expression of restriction activity following conjugal transfer by using two methods: one from colonies obtained from selection plates and the other kinetically, from bacterial cultures grown in selection broth.

First, the type I *Eco*K R-M system of *Escherichia coli* K-12 encoded on an F′ plasmid (F101-101) (10) was transferred by conjugation from donor strain JR309 (*E. coli* C *recA* Kan^r F101-101 $r^+_K m^+_K$ Tet^r) (9) to three non-K-modified recipients: an *E. coli* C strain (JR301 [C r^0m^0 Str^r]) (9) and two *E. coli* K-12 strains (NM477 Δ*hsd* $r^-_K m^-_K$ Str^r [Str^r derivative of NM477] [7] and 1228 [$r^-_K m^-_K$ Str^r] [3]). The conjugation frequencies of the crosses were approximately equivalent to those of control conjugations with an isogenic F′ plasmid (F101-102) (8) containing the genes that encode the modification methyltransferase but lacking the functional wild-type *hsdR* gene (data not shown). Upon testing the phenotypes of the conjugants (20 from each cross) obtained directly from the selection plates (L agar [8] containing streptomycin and tetracycline), we found that 59 (98%) of 60 were $r^-_K m^+_K$. Nine of these $r^-_K m^+_K$ conjugants (three from each cross) were then grown overnight in L broth (8), and the phenotype of eight (89%) of these conjugants became $r^+_K m^+_K$. Thus, most of the conjugants seem to be $r^-_K m^+_K$ immediately after conjugation but eventually they show a fully functional $r^+_K m^+_K$ phenotype.

Second, we further investigated the kinetics of expression of restriction activity after conjugal transfer. The donor for the conjugation was *E. coli* JR309, which carries the wild-type hsd_K genes on F′ plasmid F101-101 ($r^+_K m^+_K$), while the recipient was wild-type *E. coli* C Str^r strain JR301. Strain JR301 lacks R-M genes, so its DNA is not modified for the K restriction system. Single colonies of the donor and recipient strains were separately inoculated into 10 ml of L broth and grown overnight at 37°C. A 1:20 dilution was made into 10 ml of L broth, and these cultures were grown to an A_{510} of approximately 0.4 (2×10^8 cells per ml). Aliquots of 8 ml each of the donor and recipient cultures were combined and shaken gently (120 rpm) on a platform shaker at 37°C for 2 h. A 5-ml aliquot of this conjugation mixture was diluted to a final volume of 100 ml of L broth containing streptomycin (100 μg/ml) and tetracycline (20 μg/ml) for selection of conjugants. Following dilution, the A_{510} was approximately 0.1. The first samples, at zero doubling, were subjected to semiquantitative restriction spot tests as described by Colson et al. (4) and Bullas et al. (3), except that the bottom L agar contained tetracycline and streptomycin. The conjugation mixtures were further grown with maximum aeration to an A_{510} of approximately 1.0 in a 37°C shaker bath, after which they were diluted 20-fold in L broth supplemented with streptomycin and tetracycline. This dilution cycle was repeated at least 12 times. At 2-h intervals, the A_{510} values were recorded for the growing conjugant cultures and samples were removed for restriction spot tests. The number of doublings of the conjugants, n, was calculated on the basis of the A_{510} change of the conjugant culture as $n = \log (A_{510\ final}/A_{510\ initial})/\log 2$. The modification activity was measured by testing the 2-h conjugation mixture by the method of Bullas et al. (3).

Restriction activity was not detected immediately following conjugal transfer of the hsd_K genes in the population of cells grown in selective medium (Fig. 1) but was first detected after approximately 15 generations. It increased logarithmically, reaching a maximum level at approximately 30 generations after conjugation.

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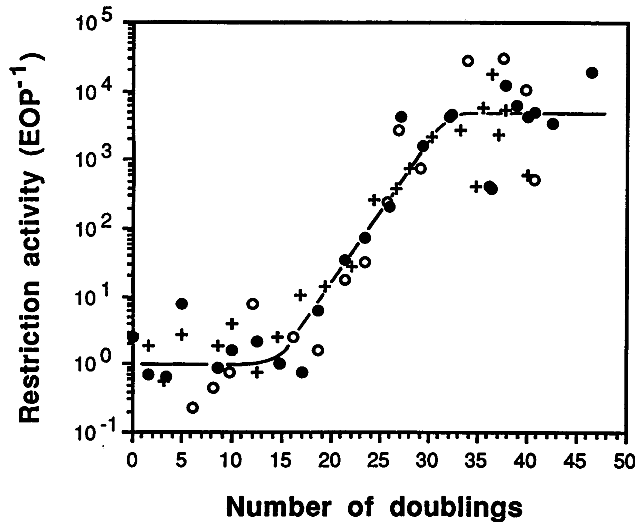

FIG. 1. Delayed expression of restriction activity (defined as the inverse of the efficiency of plating [EOP^{-1}])a following conjugal transfer of the hsd_K genes to a nonmodified strain. The three symbols shown represent results from three parallel experiments. The relative efficiency of plating of λ.0 to λ.K phage was calculated.

The modification phenotype of conjugants tested immediately following the 2-h conjugation was $m^+{}_K$ (zero doublings in Fig. 1). Modification activity was measured as the ability of phage λ grown on a lawn of the conjugation mixture to survive and lyse its host when plated on a wild-type *E. coli* K-12 strain.

Expression of the hsd_K genes may vary with the substrate of the host cell. The multifunctional restriction endonuclease modification methyltransferase complex can act upon various DNA substrates. When the cell is dividing, the normal substrate is hemimethylated DNA. When the hsd_K genes are transferred to nonmodified recipient strains, as is the case during establishment of hsd_K genes, nonmethylated DNA is the major substrate. A nonmodified recipient host provides a poor substrate for the *Eco*K methyltransferase (modification enzyme) (14) but an ideal substrate for restriction enzyme *Eco*K. If restriction activity becomes functional before the genome has been fully modified, the recipient host DNA will be digested and the host will be killed. This apparent paradox of establishing the hsd_K genes in a nonmodified recipient host can be explained by the delayed expression of the restriction activity. In this study, we have shown that there is a delay in expression of restriction activity following conjugal transfer, suggesting that the restriction activity is regulated. This regulation explains the establishment of hsd_K genes in a nonmodified recipient host. Although the precise molecular mechanism of the regulation is not known, our preliminary results from other work suggest that the control of the restriction activity is either translational or posttranslational and that the restriction activity is regulated by a gene, which we designated *hsdC*, on the chromosomes of *E. coli* C and K-12 (9). Delayed expression of the restriction activity was previously observed in the type III phage P1 R-M system (1). Furthermore, genes that control restriction activity have been reported recently for the *Pvu*II and *Bam*HI type II R-M systems: *pvuIIC* (12) and *bamHIC* (6).

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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. **Boyer, H.** 1964. Genetic control of restriction and modification in *Escherichia coli*. J. Bacteriol. **88**:1652–1660.
3. **Bullas, L. R., C. Colson, and B. Neufeld.** 1980. Deoxyribonucleic acid restriction and modification systems in *Salmonella*: chromosomally located systems of different serotypes. J. Bacteriol. **141**:275–292.
4. **Colson, C., S. W. Glover, N. Symonds, and K. A. Stacey.** 1965. The location of the genes for host controlled modification and restriction in *Escherichia coli* K-12. Genetics **52**:1043–1050.
5. **Fuller-Pace, F. V., G. M. Cowan, and N. E. Murray.** 1985. *Eco*A and *Eco*E: alternatives to the *Eco*K family of type I restriction and modification systems of *Escherichia coli*. J. Mol. Biol. **186**:65–75.
6. **Ives, C. L., P. D. Nathan, and J. E. Brooks.** 1992. Regulation of the *Bam*HI restriction-modification system by a small intergenic open reading frame, *bamHIC*, in both *Escherichia coli* and *Bacillus subtilis*. J. Bacteriol. **174**:7194–7201.
7. **Murray, N. E., J. A. Gough, B. Suri, and T. A. Bickle.** 1982. Structural homologies among type I restriction-modification systems. EMBO J. **1**:535–539.
8. **Prakash, A., B. Valinluck, and J. Ryu.** 1991. Genomic *hsd*-Mu(*lac*) operon fusion mutants of *Escherichia coli* K-12. Gene **99**:9–14.
9. **Prakash-Cheng, A., S. S. Chung, and J. Ryu.** The expression and regulation of hsd_K genes after conjugal transfer. Mol. Gen. Genet., in press.
10. **Ryu, J., P. T. Rajadas, and L. R. Bullas.** 1988. Complementation and hybridization evidence for additional families of type I DNA restriction and modification genes in *Salmonella* serotypes. J. Bacteriol. **170**:5785–5788.
11. **Suri, B., and T. A. Bickle.** 1985. *EcoA*: the first member of a new family of type I restriction modification systems. Gene organization and enzymatic activities. J. Mol. Biol. **186**:77–85.
12. **Tao, T., J. C. Bourne, and R. M. Blumenthal.** 1991. A family of regulatory genes associated with type II restriction-modification systems. J. Bacteriol. **173**:1367–1375.
13. **Wilson, G. G., and N. E. Murray.** 1991. Restriction and modification systems. Annu. Rev. Genet. **25**:585–627.
14. **Yuan, R., and D. 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.

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