

## Restriction Endonucleases R.EcoKI and R.EcoR124I Are Probably Located in Different Environments within the Bacterial Cell

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**ABSTRACT.** We describe the phenomenon of a transient state of R124I restriction deficiency after long-term storage of the *E. coli*[pCP1005] strain at 4 °C, or after growth of the culture in synthetic M9 medium with the nonmutagenic solvent dimethyl sulfoxide. The unusual high reversion from the R<sup>+</sup><sub>124</sub> to the R<sup>-</sup><sub>124</sub> phenotype was observed only in *E. coli* strain transformed with the high-copy number plasmid pCP1005 carrying *EcoR124I* *hsdR*, M and S genes cloned, but not with strains carrying the natural conjugative plasmid R124. The effect of both treatments on the expression of *EcoR124I* phenotype in relation to the possible location of R.EcoR124I restriction endonuclease in *E. coli* is discussed.

In order to understand the function of a gene it is necessary to isolate specific mutations with an observable phenotype, to localize these mutations and characterize the precise amino acid changes produced in the protein. This type of genetic analysis presupposes that the phenotype observed will be unaffected by the location of the given gene either on a plasmid or on the bacterial chromosome, or by the physiological state of the bacteria. However, this is certainly not always the case.

We attempted to isolate temperature-sensitive mutants in the type IC host specificity (*hsd*) *EcoR124I* restriction-modification system. Such mutants are thought to be affected in their DNA recognition properties or in the assembly of the HsdRMS subunits into an active endonuclease. The method of isolation was, in principle, the same as that described and used with the *EcoKI* system by Hubáček and Glover (1970), including ethyl methanesulfonate mutagenesis, two-step enrichment of R<sup>-</sup> mutants at 42 °C and one-step enrichment of R<sup>+</sup><sub>ts</sub> mutants at 30 °C. The bacterial strain used in these experiments with *EcoR124I* was *E. coli* CR<sup>-</sup>M<sup>-</sup> carrying the plasmid pCP1005 with the *EcoR124I* *hsdR*, M and S genes as cloned by Firman *et al.* (1985).

An unexpected result was obtained, following EMS mutagenesis of the cell population, in that the EOP of  $\lambda$ *vir*.K increased from about 10<sup>-6</sup> to 10<sup>-1</sup>. This shift from the R<sup>+</sup><sub>124</sub> to the R<sup>-</sup><sub>124</sub> phenotype was also found after long-term (several weeks) storage of the *E. coli* C[pCP1005] strain at 4 °C, or after growth of the culture in synthetic M9 medium in the presence of the nonmutagenic solvent dimethyl sulfoxide (Me<sub>2</sub>SO) (Fig. 1, curve 1). The *EcoR124I* modification phenotype was not significantly changed under these conditions (Table I).

When the R<sup>-</sup><sub>124</sub>M<sup>+</sup><sub>124</sub> overnight culture thus obtained, was diluted in bacterial buffer by 5 ppm and 0.1 mL of this dilution was inoculated (in analogy with the Max-Delbrück fluctuation test) into 5 mL LB with 100 mg/L of ampicillin, out of 20 inoculated tubes only 12 cultures grew and in 8 tubes no growth was observed. All the growing cultures, which were derived with a high probability from a single cell, were found to be of the R<sup>+</sup><sub>124</sub>M<sup>+</sup><sub>124</sub> phenotype (EOP of  $\lambda$ *vir*.K was about 10<sup>-6</sup>). The same shift from the R<sup>-</sup><sub>124</sub> to the R<sup>+</sup><sub>124</sub> phenotype was also observed when 0.1 mL of the R<sup>-</sup><sub>124</sub>M<sup>+</sup><sub>124</sub> overnight culture, diluted to 10<sup>-5</sup>, was spread on LA with Ap and grown overnight at 37 °C. All 125 inoculated colonies, grown subsequently overnight in LB, were found to be of the R<sup>+</sup><sub>124</sub> phenotype. That is, the clones growing from a single cell revert back to the restriction-proficient phenotype.

This phenomenon complicated our ability to isolate temperature-sensitive mutations in the *EcoR124I* *hsd* genes. In an attempt to overcome these problems a number of different genetic backgrounds were used for expression of the *EcoR124I* R-M phenotype. In *E. coli* QR47R<sup>+</sup>κM<sup>+</sup>κ [pCP1005] clones the *EcoR124I* restriction phenotype was expressed in freshly prepared transformants at a low level only (EOP of  $\lambda$ *vir*.K was about 10<sup>-1</sup>) and was sensitive to Me<sub>2</sub>SO (Fig. 1, curve 5); the *EcoR124I* modification phenotype was expressed normally. In this R<sup>-</sup><sub>124</sub>M<sup>+</sup><sub>124</sub> strain the *EcoKI* system functioned normally and was not influenced by cultivation in M9 medium with Me<sub>2</sub>SO (Fig. 1,

curve 4). In *E. coli* C600R<sup>+</sup>κM<sup>+</sup>κ[pCP1005] under the conditions where the *EcoR124I* restriction was not functioning (10% Me<sub>2</sub>SO) the *EcoKI* restriction was normal (Table I). The *EcoKI* phenotype was not sensitive to Me<sub>2</sub>SO even when the *EcoKI* *hsd* gene complex was cloned in the high-copy number plasmid pVMC3 (Fig. 1, curve 2), constructed by Weiserová *et al.* (1993). These results strongly suggest that the increase in the EOP of λvir.K when grown on a strain producing *EcoR124I* was dependent upon the physiological state of the bacteria and was not caused by the appearance of true and stable *EcoR124I* restriction deficient mutants.

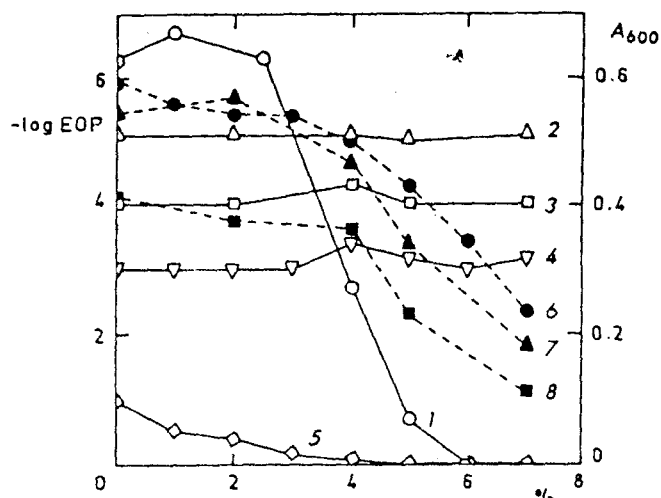


Fig. 1. The effect of Me<sub>2</sub>SO (%) on the expression of the restriction phenotypes (log EOP of phage) of *EcoKI* and *EcoR124I* in *E. coli* strains harboring different plasmids. The strains used are *E. coli* C R<sup>-</sup>M<sup>-</sup> (Bertani and Weigle 1953) and *E. coli* QR47 R<sup>+</sup>κM<sup>+</sup>κ (Signer and Weil 1968). Restriction was scored with phages λvir.O, λvir.K and λvir.K,124. Restriction specificity R124 (curves 1, 3 and 5) indicates the EOP of λvir.K relative to λvir.K,124; restriction specificity K (curves 2 and 4) indicates the EOP of λvir.O relative to λvir.K under the condition when *EcoR124I* restriction is not functioning efficiently (EOP of λvir.K is 0.1–1); log EOP of phage is the efficiency of plating of λvir.K or λvir.O on cell culture grown in M9 medium with varying concentrations of Me<sub>2</sub>SO.

Bacterial concentration is expressed as absorbance of cultures at 600 nm (*A*<sub>600</sub>); 1 – *E. coli* C[pCP1005]; 2 – *E. coli* C[pVMC3]; 3 – *E. coli* C[R124]; 4 – restriction phenotype *EcoKI* in *E. coli* QR47 R<sup>+</sup>κM<sup>+</sup>κ[pCP1005] strain; 5 – restriction phenotype *EcoR124I* in *E. coli* QR47 R<sup>+</sup>κM<sup>+</sup>κ[pCP1005] strain; 6–8 (dashed lines) – bacterial density of cultures *E. coli* C[pCP1005], *E. coli* C[pVMC3] and *E. coli* C[R124], respectively.

The phenomenon of a transient state of restriction deficiency as observed in *E. coli* C and the other *E. coli* strains tested (*i.e.* QR47 and C600), when transformed with the high-copy number plasmid pCP1005, was in contrast to that observed with *E. coli* C carrying the natural conjugative plasmid R124 (Hedes and Datta 1972; Pritchard and Rowbury 1980) from which the *EcoR124I* *hsd* genes were cloned (Fig. 1, curve 3). In this case the *EcoR124I* restriction phenotype was not sensitive to Me<sub>2</sub>SO and produced an efficiency of plating of λvir.K by two orders of magnitude lower than obtained with *E. coli* C[pCP1005] (compare curves 1 and 3 in Fig. 1). This could be explained by a higher level of restriction endonuclease in the cell produced by the high-copy number plasmid pCP1005.

Dimethyl sulfoxide is a dipolar aprotic surfactant, an inducer of differentiation of a wide variety of cells, such as Friend leukemia cells, human promyelocytic leukemia cells and others (cited in Tapiero *et al.* 1983) and exerts a positive effect on production (excretion) of amino acids in bacteria

Table I. *EcoKI* and *EcoR124I* R-M phenotypes of *E. coli* C600[pCP1005] after 16 h of cultivation in the M9 medium with Me<sub>2</sub>SO

Me <sub>2</sub> SO %	Restriction <sup>a</sup>		Modification <sup>b</sup>	
	K	124	Fraction of phage λ plating on strain specificity:	
V/V	λvir.O	λvir.K	K	124
0	–	10 <sup>-6</sup>	–	0.7
5	–	0.1	–	0.5
7	2 × 10 <sup>-4</sup>	0.4	1	0.4
10	5 × 10 <sup>-4</sup>	1	0.8	0.3

<sup>a</sup>See the footnote below Fig. 1. Strain *E. coli* C600 is described in Appleyard (1954).

<sup>b</sup>Modification specificity K indicates the EOP of phage λ produced by the tested strain measured on *E. coli* K and C indicator strains. Modification specificity 124 indicates the EOP of phage λ produced by the tested strain measured on *E. coli* C[pCP1005] and *E. coli* C indicator strains.

(Tang *et al.* 1989). As concluded by Tapiero *et al.* (1983), the effect of Me<sub>2</sub>SO is related to the composition and organization of the cell membrane components. The influence of low temperature and Me<sub>2</sub>SO on the R-M phenotype may be related to the effect of both treatments on the structure and properties of the bacterial membrane. The concentrations of Me<sub>2</sub>SO which have the apparent effect on the expression of EcoR124I restriction are relatively high and could have some detrimental effects on bacteria. In reality, however, no relationship was observed between the effect of Me<sub>2</sub>SO on bacterial density and the sensitivity of R124I restriction to the gradually increasing concentrations of the compound. The rate of decline in the bacterial density of the cultures with increasing concentration of Me<sub>2</sub>SO, is the same for EcoR124I (Fig. 1, curves 6 and 8) and EcoKI (Fig. 1, curve 7). It seems that it is a specific effect of this compound on the expression of EcoR124I restriction in *E. coli* when transformed with the high-copy number plasmid pCP1005. A 1000-fold increase in the EOP of λvir.K was observed in those cultures grown in 4% Me<sub>2</sub>SO, and under such conditions the decrease in bacterial density was not so evident. It is possible that the R.EcoKI and R.EcoR124I restriction endonucleases are bound to different receptors within the bacterial membrane and that this could affect the sensitivity of the R-M phenotype to different physiological states. One would have to propose that the R.EcoR124I endonuclease is bound to the membrane not so firmly as R.EcoKI and is easily released and physiologically inactivated. The evolutionary significance of these different effects of bacterial physiology on the R-M phenotype of EcoR124I and EcoKI may lie in the fact that EcoR124I is a plasmid-borne system and, following conjugation, the expression of restriction before modification must be tightly regulated. This control may involve activation of the endonuclease only following transport to its correct cell location. This would allow the modification methylase to accomplish methylation of the host chromosome. It will be interesting to make a similar investigation of the effect of bacterial physiology on the other plasmid-born type I R-M system EcoDXXI (Piekarowicz *et al.* 1985).

However, the detection of the EcoR124I restriction phenotype depends not only on the location of *hsd* genes, but also on the copy number. After cloning to a multicopy plasmid, the R124I restriction phenotype is sensitive to the above mentioned treatments. In the case of the natural location of the *hsd* genes on the R124 plasmid no changes in the level of R124I restriction are observed. We would assume that after cloning the EcoR124I *hsd* genes some genetic element(s) is lost which, in the natural R124 plasmid, was selected during the evolution of the cell harboring the plasmid restriction system to support the expression of the restriction phenotype. The product of this element could affect the binding of the restriction enzyme to some cell membrane component. It is interesting to note that the R124 conjugative plasmid has been found to decrease the level of the OmpF protein in the cell, and to alter the sensitivity of the bacterial cells to a wide range of agents in OmpC<sup>-</sup> mutants (Rossouw and Rowbury 1984). There may be some relationship between OmpF production and the cellular localization of the EcoR124I endonuclease.

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