



The Alternate Expression of Two Restriction and Modification Systems

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Summary. Plasmids R124 and R124/3 carry genes coding for two different R-M systems and normally only one set of genes is expressed. These genes can be translocated to F plasmids that are compatible with the R factors and in strains carrying these F plasmids and an R factor a *trans*-acting regulatory mechanism switches off the expression of R-M genes on the introduced plasmid. Additionally the unexpressed genes on the introduced plasmid are expressed. The regulatory mechanism controlling the alternative expression of R124 and R124/3 R-M genes involves a physical rearrangement of DNA sequences.

Introduction

The plasmid R124 is a large conjugative plasmid in incompatibility group F IV and confers resistance to tetracycline (Hedges and Datta 1972). It codes for a unique restriction and modification (R-M) system capable of restricting the growth of bacteriophage λ and a number of other phages (Bannister and Glover 1968). Non-restricting mutants of this plasmid have been isolated and characterized and they fall into two phenotypic classes, r^-m^+ and r^-m^- which were obtained in roughly equal numbers (Bannister and Glover 1970). A derivative plasmid has been obtained which expresses a R-M system with a different DNA specificity from R124. This derivative plasmid was designated R124/3 (S. Hughes pers. comm.) and this designation is also used to define the R-M system expressed by R124/3.

In this paper we present evidence that genes coding for each R-M system are carried by both R124 and R124/3 plasmids and that normally only one set of genes is expressed. The mechanism that ensures that only one set of genes is expressed was investigated in a series of experiments in which a plasmid expressing the genes for the R124 R-M system and a plasmid expressing the genes for the R124/3 R-M system were both introduced into the same cell. These experiments were made feasible by the recent discovery that the R-M genes from R124 and R124/3 can be translocated to F plasmids that are compatible with the R factors. (Glover and Firman (1982) and unpublished observations). Evidence is also presented for a *trans*-acting regulatory mechanism coded for by a resident plasmid that can switch off the expression of R-M genes on an introduced plasmid and for a *trans*-acting regulatory mechanism that can switch

on the expression of an alternative set of R-M genes carried by the introduced plasmid.

The regulatory mechanism controlling the alternate expression of R124 and R124/3 R-M genes involves a physical rearrangement of DNA sequences.

Methods

Bacterial Strains, Bacteriophages and Plasmids. The *Escherichia coli* K12 strains, bacteriophages and plasmid used in this study are listed in Table 1.

Table 1. Bacterial strains, plasmids and bacteriophages

	Relevant genotype	Source
<i>Bacterial strains</i>		
C600	<i>thr leu thi lac str-r</i>	Appleyard (1954)
C600-1	<i>thr leu thi lac nal-r</i>	This laboratory
AB1157	<i>thr leu thi pro arg his lac str-r</i>	A.J. Clarke
AB2463	<i>thr leu thi pro arg his lac str-r recA</i>	A.J. Clarke
2K	<i>thi serB lac str-r</i> $r_K^- m_K^-$	This laboratory
J53-1	<i>pro met nal-r</i>	N. Datta
<i>Plasmids</i>		
R124		Hedges and Datta (1972)
R124/3		S. Hughes
F ⁺		This laboratory
F' <i>lac</i> ⁺		This laboratory
F' <i>lac</i> ⁺ r ₁₂₄ ⁺ m ₁₂₄ ⁺		This paper
F' <i>lac</i> ⁺ r _{124/3} ⁺ m _{124/3} ⁺		This paper
F ⁺ r ₁₂₄ ⁺ m ₁₂₄ ⁺		This paper
R124::Tn7(3)		This paper
<i>Bacteriophages</i>		
λ vir		Jacob and Wollman (1954)
MS2		This laboratory
T7		This laboratory

Media. Nutrient media, minimal media and dilution buffers were as described by Hubacek and Glover (1970).

Chemicals. Amino acids were from Sigma and used at final concentrations of 10 µg/ml. Antibiotics were used at the following concentrations: streptomycin (25 µg/ml), tetracycline (50 µg/ml). Restriction endonucleases were from Boehringer.

Buffers for Plasmid DNA Isolation. 25% w/v sucrose dissolved in 0.05 M Tris/HCl pH 8.0; lysozyme 5 mg/ml in 0.25 M Tris/HCl pH 8.0; 0.25 M disodium EDTA pH 8.0; lytic mix was 1% Triton X-100 in 0.05 M Tris/HCl and 0.06 M disodium EDTA pH 8.0.

Other Buffers. TES: 50 mM Tris/HCl; 5 mM disodium EDTA; 50 mM NaCl; pH 7.5. TEN: 10 mM Tris/HCl; 1 mM disodium EDTA; 50 mM NaCl; pH 7.5. Isopropanol/TEN/CsCl: propan-2-ol saturated with a saturated solution of CsCl in TEN buffer, only the upper layer was used. TMN: 10 mM MgCl₂; 5 mM mercaptoethanol; 5 mM NaCl; 10 mM Tris/HCl; pH 7.5. Buffer E: 40 mM Tris; 20 mM sodium acetate; 1 mM disodium EDTA; adjusted to pH 8.2 with acetic acid. Sample buffer: 10% w/v Ficoll; 0.5% SDS in buffer E; 0.01%–0.1% w/v bromophenol blue.

Tests for Restriction and Modification. The accurate and spot-test methods described by Colson et al. (1965) were used throughout.

Plasmid Transfer. Plasmid transfer was carried out by mixing 1 ml of an overnight culture of the donor strain with 1 ml of the recipient strain and 5 ml of fresh pre-warmed nutrient broth in a 50 ml Erlenmeyer flask. The mixture was shaken gently for 1 h at 37° C.

Plasmid Curing. R124 and R124/3 were cured following the method of Hahn and Ciak (1972) using 10⁻⁴ M ethidium bromide in broth (pH 7.0) at 37° C. After overnight incubation in ethidium bromide broth curing was detected by replica-plating colonies from nutrient agar plates to nutrient agar plates containing tetracycline. F and F prime plasmids were cured using 10⁻⁴ M acridine orange at pH 7.1. Curing was detected by loss of MS2 sensitivity for F or by loss of the Lac⁺ phenotype for strains containing F' lac⁺ plasmids.

Isolation of Plasmid DNA. Plasmid DNA isolation was based on the isopycnic centrifugation technique of Radloff et al. (1967) using cleared lysates.

Restriction Endonuclease Digestion of Plasmid DNA and Agarose Gel Electrophoresis. 3–5 µg of plasmid DNA was used for each well in the gel electrophoresis. 10 units of the appropriate restriction endonuclease in TEN buffer was added and the digestion carried out at 37° C for 45 min. The reaction was stopped by heating at 65° C for 10 min. 5 µl of sample buffer was added and the whole sample loaded into the well of the agarose gel. Gels were prepared by dissolving agarose (0.7–1.4%) in buffer E boiled for 10 min and kept molten until use at 60° C. Gels were run on a Biorad Protean 16 vertical slab apparatus or a Bethesda Research Labs horizontal gel apparatus at a constant

voltage of 3.5 volts cm⁻¹ overnight. Gels were stained in buffer E containing 0.5 µg/ml ethidium bromide, visualized in a Chromavue ultra-violet cabinet and photographed with a Zenith E camera using Ilford HP5 film with a red filter.

Results and Discussion

Characterization of R124 and R124/3 R-M Systems

Table 2 shows the approximate efficiency of plating of phage λ on *E. coli* strains carrying the plasmids R124 and R124/3. It is quite clear from these results that the two specificities are completely different in that R124 restricts phage λ.K12 and λ.R124/3 equally and R124/3 restricts λ.K12 and λ.R124 with equal efficiency. These two R-M systems are quite distinct from other *E. coli* R-M systems and other plasmid or phage-coded systems (Bannister and Glover 1968, 1970 and unpublished observations).

Genes for Both R-M Systems are Carried by R124 and R124/3

Evidence that genes for both R-M systems are carried by R124 and by R124/3 was first obtained by S. Hughes (personal communication) who observed that, at a low frequency (approximately 10⁻⁷), the R-M specificity of the plasmid R124 switched spontaneously to that of R124/3 and that with a similar low frequency R124/3 R-M specificity switched to R124.

In the course of characterizing restriction-deficient mutants of both R124 and R124/3 we have isolated non-restricting mutants which are nevertheless able to express both modifications (Glover 1980). These mutants are designated r⁻m₁₂₄⁺m_{124/3}⁺ in Table 3 and will be discussed in a subsequent publication.

Acquisition of R124 and R124/3 R-M Genes by IncFI Plasmids

When F, or its F-prime derivatives, are introduced into bacteria carrying R124 or R124/3, we have observed that a number of molecular rearrangements take place, all of

Table 2. Plating efficiencies of phage λ on *E. coli* K12 strains carrying R124 or R124/3 plasmids

Phages	Bacterial strains		
	K12	K12 (R124)	K12 (R124/3)
λ.K12	1.0	1 × 10 ⁻⁴	1 × 10 ⁻³
λ.R124	1.0	1.0	1 × 10 ⁻³
λ.R124/3	1.0	1 × 10 ⁻⁴	1.0

Table 3. R-M phenotype of restriction-deficient mutants of plasmids R124 and R124/3

Plas- mid	Num- ber of r ⁻ mu- tants	Modification phenotype				
		m ₁₂₄ ⁺	m ₁₂₄ ⁻	m _{124/3} ⁺	m _{124/3} ⁻	m _{124, 124/3} ⁺
R124	54	33	15	—	—	6
R124/3	62	—	—	31	23	8

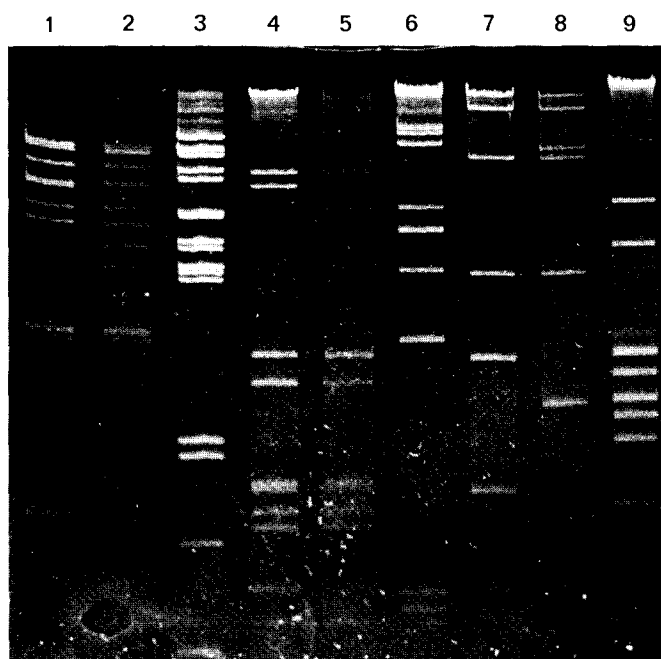


Fig. 1. Agarose gel electrophoresis of *EcoRI*, *HindIII* and *BamHI* digests of F, $Fr_{124}^+m_{124}^+$ and R124 DNA. Wells 1, 4 and 7 F DNA; wells 2, 5 and 8 $Fr_{124}^+m_{124}^+$ DNA; wells 3, 6 and 9 R124 DNA. *EcoRI* digests wells 1, 2 and 3; *HindIII* digests wells 4, 5 and 6; *BamHI* digests wells 7, 8 and 9. 1% agarose gels electrophoresed at 3.5 volts/cm for 16 h

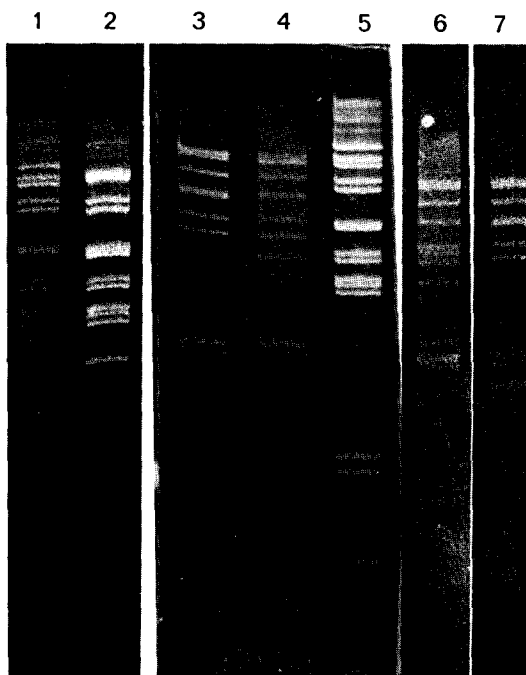


Fig. 3. Agarose gel electrophoresis of *EcoRI* restriction enzyme digestion of plasmids switched in the expression of R-M specificity genes. Well 1 R124; well 2 R124/3; well 3 F^+ ; well 4 $Fr_{124}^+m_{124}^+$; well 5 R124; well 6 $Fr_{124/3}^+m_{124/3}^+$; well 7 F^+ . 1% agarose gels electrophoresed at 3.5 volts/cm for 16 h

which are *recA* independent, and therefore, involve illegitimate recombination events. The details of these rearrangements will be the subject of a separate communication and in this section we will deal only with the acquisition of R-M genes by F plasmids.

Previously isolated strains carrying F and R124 were tested for restriction and modification and those strains which subsequently scored r^+m^+ were then cured of R124 using ethidium bromide as described in Methods. After curing the strain was plated out and individual colonies were tested for tetracycline sensitivity to confirm the elimi-

nation of R124 and for MS2 sensitivity to confirm the presence of F. Strains carrying F were then tested for restriction and modification and the F factor was transferred to another strain. All the F factors were transfer proficient and after transfer the strains were again tested for restriction and modification.

Of 19 cultures cured of R124 and subsequently tested for restriction and modification in this way all were found to contain F plasmids which had acquired the R124 R-M genes and were able to transfer them to F^- recipients. The plasmid DNA from several of these $F^+r^+m^+$ strains was

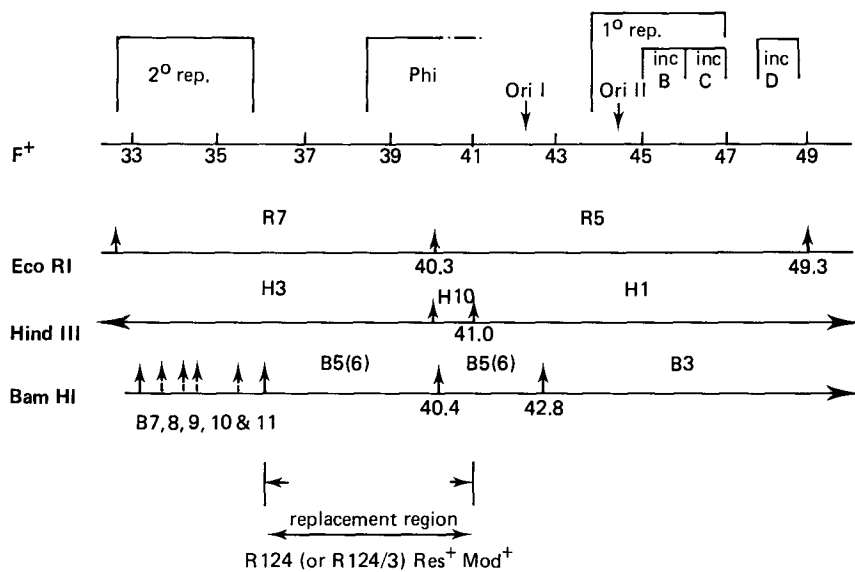


Fig. 2. Diagram to show the region of F DNA altered by the acquisition of R124 and R124/3 restriction and modification genes

isolated and cleaved by restriction endonuclease digestion and the resulting fragments separated by agarose gel electrophoresis. All $F^+r_{124}^+m_{124}^+$ plasmids were identical and Fig. 1 shows the results of *EcoRI*, *HindIII* and *BamHI* digestion of these plasmid molecules and F DNA as a control. The *HindIII* and *BamHI* digests of $F^+r_{124}^+m_{124}^+$ show loss of more than one F DNA fragment which suggests that the translocation of the restriction genes to F is not a simple transposition but involves the loss of some F DNA as well as the acquisition of DNA from R124. The *BamHI* digest of $F^+r_{124}^+m_{124}^+$ shows a net gain of approximately 10 kilo bases of DNA. The region of F which is involved includes the *BamHI* fragments 5 and 6, the *HindIII* fragments 3 and 10 and the *EcoRI* fragment 5. It will be necessary to identify by Southern blotting which segments of F have been lost and which segments of R124 have been gained by these $F^+r_{124}^+m_{124}^+$ plasmids before their molecular structure and the mechanism that generates them can be understood. However, the mechanism is known to be a *recA* independent event. Figure 2 represents diagrammatically the loss of segments of F and the addition of segments of DNA carrying the R-M genes from R124. Consistent with this loss of segments of F DNA is the loss of the phage inhibition property (*phi*) tested using phage T7, from all $F^+r^+m^+$ plasmids.

Similar results (Fig. 3) have been obtained with the plasmids R124/3 and F. The structure of all $F^+r_{124/3}^+m_{124/3}^+$ plasmids was the same. However, $F^+r_{124}^+m_{124}^+$ and $F^+r_{124/3}^+m_{124/3}^+$ shows certain characteristic differences as might be expected from the differences in physical structure of R124 and R124/3 (see Fig. 3).

Control of the Expression of R124 and R124/3 R-M Specificity in Strains Carrying two Plasmids and Evidence for a Switch in the Expression of R-M Genes

Table 4 shows the R-M phenotypes of strains constructed to carry either R124 or R124/3 and derivatives of $F' lac^+$ which had acquired the R-M genes as described in the previous section. It is clear from these results that these strains do not express both R-M systems as had been expected but only one, namely that of the resident plasmid. In each case the R-M specificity coded by the introduced plasmid is not expressed. We therefore conclude that the resident plasmid has a *trans*-acting regulatory mechanism capable of switching off the expression of R-M genes on an introduced plasmid. It is likely also that the same regulatory mechanism is responsible for the fact that the resident plasmid expresses only one of the two alternative sets of R-M genes which it carries.

The strains constructed to carry two plasmids, one an R factor and the other an $F' lac^+$ plasmid into which R-M genes had been inserted, were then cured of either the R plasmid or the F plasmid.

Strains cured of the introduced plasmid which was no longer expressing its R-M genes showed, as expected, the R-M phenotype of the resident plasmid (data not shown).

However, strains cured of the resident plasmid did not show the R-M phenotype of the introduced plasmid. In each case the R-M phenotype was switched to the alternative one. For example, when R124 was introduced into a strain carrying $F' lac^+r_{124/3}^+m_{124/3}^+$ and the strain was subsequently cured of the F' plasmid it displayed the R-M specificity characteristic of R124/3 indicating that expression of

Table 4. R-M phenotype of *E. coli* strains constructed to carry both R124 and R124/3 R-M specificity systems

Plasmid in donor strain	Plasmid in recipient	R-M phenotype of transconjugants containing both plasmids
R124	$F' lac^+r_{124/3}^+m_{124/3}^+$	$r_{124/3}^+m_{124/3}^+$
R124/3	$F' lac^+r_{124}^+m_{124}^+$	$r_{124}^+m_{124}^+$
$F' lac^+r_{124}^+m_{124}^+$	R124/3	$r_{124/3}^+m_{124/3}^+$
$F' lac^+r_{124/3}^+m_{124/3}^+$	R124	$r_{124}^+m_{124}^+$

the R-M genes on the introduced plasmid had been switched from R124 to R124/3. This switch in the expression of R-M genes occurred in all four of the experiments illustrated in Table 5 and since it occurred in a *recA* background is unlikely to be due to a simple exchange of alleles between the resident and introduced plasmids.

Further confirmation of this was obtained in an experiment utilizing a plasmid provided by W.A. Creasey. This plasmid R124::Tn7(3) was obtained by Tn7 insertion into the RM genes of R124 and has a $r_{124}^+m_{124}^+$ phenotype. When this plasmid was transferred into a strain carrying $F' lac^+r_{124/3}^+m_{124/3}^+$ and subsequently retested after curing of the resident F-prime, it was found to have the phenotype $r_{124/3}^+m_{124/3}^+$. The plasmid still conferred trimethoprim resistance characteristic of Tn7. Thus the switch in specificity observed could not be the result of reciprocal recombination between the two plasmids which would have resulted in the loss of the inserted Tn7.

Molecular Basis of the Switch in the Expression of R-M Genes

Preliminary evidence (Glover 1980) led us to believe that the switch in the expression of R-M genes was accompanied by a rearrangement of DNA sequences on the plasmid.

In an attempt to understand the molecular basis of this switch mechanism the DNA of R124 and the switched plasmid R124/3 was isolated and digested with *EcoRI* and the fragments separated by agarose gel electrophoresis. Figure 3 shows that there is a molecular rearrangement involved when R124 is switched to R124/3. Fragments 1 and 3 of R124 are replaced by three additional fragments 2a, 6b and 12a. Similarly a rearrangement is observed when $F' lac^+r_{124}^+m_{124}^+$ is switched in the expression of R-M genes to $F' lac^+r_{124/3}^+m_{124/3}^+$ and all isolates of switched plasmids were found to have the same *EcoRI* digestion pattern. In the absence of a complete restriction fragment map of the plasmids R124 and R124/3 it is difficult to explain the relationship between these rearrangements and the alternate expression of R-M genes. That they are related is borne out by the fact that F plasmids carrying R-M genes can be alternately switched from R124 to R124/3 specificity and *vice versa* at will by repeatedly performing the experiments described in the previous section.

The simple inversion of a segment of DNA such as that associated with the switch in expression of *Salmonella* flagella antigens (Zeig et al. 1977) will not account for the physical differences we have observed between R124 and R124/3 when gene expression is switched. Alternatively, the

Table 5. Switch in the expression of plasmid ^a-coded R-M specificity

R-M specificity of donor plasmid	R-M specificity of recipient plasmid	R-M specificity of transconjugants cured of resident plasmid
(R124) $r_{124}^+ m_{124}^+$	(F') $r_{124/3}^+ m_{124/3}^+$	(R124) $r_{124/3}^+ m_{124/3}^+$
(R124/3) $r_{124/3}^+ m_{124/3}^+$	(F') $r_{124}^+ m_{124}^+$	(R124/3) $r_{124}^+ m_{124}^+$
(F') $r_{124}^+ m_{124}^+$	(R124/3) $r_{124/3}^+ m_{124/3}^+$	(F') $r_{124/3}^+ m_{124/3}^+$
(F') $r_{124/3}^+ m_{124/3}^+$	(R124) $r_{124}^+ m_{124}^+$	(F') $r_{124}^+ m_{124}^+$

^a The plasmids used in these experiments are those listed in Table 4

cassette model advanced to explain the switch in expression of mating type genes in *Saccharomyces cerevisiae* (Hicks et al. 1979) may form the basis for this genetic switch mechanism for the expression of genes controlling DNA specificity. Experiments are in progress to clone this region of the plasmid which should permit the elucidation of the molecular basis of this switch.

Acknowledgement. G. Watson and C. Price thank the Medical Research Council for the tenure of Research Studentships.

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Communicated by J. Schell

Received July 13, 1982 / January 10, 1983