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R Factor-Controlled Restriction and Modification of Deoxyribonucleic Acid: Restriction Mutants

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Restriction mutants of two different R factor-controlled host specificities (RI and RII) were isolated. All of the restriction mutants examined had a normal modification phenotype. No complementation was observed between the RI and RII host specificities. It is concluded that for each host specificity no protein subunit is shared by the restriction endonuclease and modification methylase.

Restriction endonucleases and modification methylases are controlled by chromosomal, plasmid, or viral genes (for reviews see 4, 10). At the present time seven different host specificities are known in *Escherichia coli* strains, K, B, A, 15, P1, RI, and RII (5-7; Slocum and Boyer, *unpublished data*; and *see below*). The host specificity of a given organism is determined by the sequence of deoxynucleotide base pairs (substrate) recognized by the organism's restriction endonuclease and modification methylase. Usually, the strain designation of the organism is used to identify the host specificity, e.g., K and B host specificities for *E. coli* strains K-12 and B. Thus, deoxyribonucleic acid derived from *E. coli* B acts as a substrate for the K endonuclease and methylase and vice versa. The *E. coli* K-12, B, A, and *Salmonella typhimurium* restriction and modification enzymes are controlled by chromosomal alleles located near the *serB* locus (8, 11, 12, 17; Slocum and Boyer, *unpublished data*). The restriction and modification enzymes of the phage P1 and a related plasmid, 15, are controlled by alleles (5), and some R factors of both types (fi^+ and fi^-) also control restriction and modification enzymes (6, 7, 21, 22).

Restriction mutants of the K-12 and B strains are more or less equally divided between normal and mutant modification function (23). The explanation for this mutant distribution is that there are three cistrons controlling these enzymes. The restriction and modification enzymes of these strains have at least one protein subunit in common which is involved in recognition of the substrate (10, 15). There is also some evidence that the restriction endonucleases and modification methylases of these strains have two different protein subunits in common, and that the methylase containing two of the protein subunits serves as a "core" protein for the endonuclease which is constructed by the addition of a third protein subunit (16). This model is in keeping with the large molecular weights reported for the B and K restriction endonucleases (18, 19; Yoshimori, Roulland-Dussoix, Aldridge, and Boyer, *unpublished data*). The same mutant distribution has been reported for the P1 (15), 15, and A (5), and the RI and RII host specificities (7). The recovery of r^-m^+ and r^-m^- mutants of these host specificities might be interpreted in terms of the three cistrons and subunit structures defined for the K and B restriction and modification enzymes. However, we present genetic evidence in this paper and enzymological evidence in subsequent papers (Yoshimori, Roulland-Dussoix, Aldridge, and Boyer, *unpublished;* Yoshimori, Roulland-Dussoix, Goodman, and Boyer, *unpublished*) that the genetic basis and subunit construction of the RI and RII restriction endonucleases and modification methylases are fundamentally different from the K and B enzymes.

MATERIALS AND METHODS

Nomenclature. The restriction and modification host specificities of the fi^+ and fi^- R factors will be referred to as RI and RII, respectively. The modification of phage stocks are designated as λ·RI, λ·RII,

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etc.; the restriction and modification phenotypes are designated as r_{RI}^+ m_{RI}^+, etc.

Bacteria and phages. The principal bacterial strains used in this study were *E. coli* K-12 (W3550) r_K^+ m_K^+; *E. coli* C (HB81); *E. coli* K-12 1100 r_B^+ m_B^+ (HB129) (19). The male-specific phage fd, λvir ($cb_2, v_2 v_1 v_3$) were the bacteriophages used in this study.

The *fi*$^-$ plasmid was obtained from W. Arber and was originally identified as R15 by Watanabe (21). This plasmid carries genes for streptomycin and sulfonamide resistance. The original *fi*$^+$ plasmid (obtained from a clinical isolate), carrying the RI host specificity genes, was resistant to ampicillin, tetracycline, streptomycin, and sulfonamide. However, after this plasmid was transferred to several strains, the Amp^r Tet^r phenotype was lost along with the ability to promote transfer. The original isolate suffered the same fate.

An *fi*$^+$ plasmid derepressed for pili synthesis and carrying genes for resistance to streptomycin, sulfonamide, chloramphenicol, and ampicillin (R1drd obtained from R. Silver) was crossed to a His$^-$ Nal^r r_K^+ m_K^+ strain, carrying the defective plasmid. Selection was made from Nal^r Amp^r clones, and they were screened for RI restriction activity. These clones were crossed to a His$^+$ Amp^s strain (W3550), and His$^+$ Amp^r clones were selected. The RI host specificity genes were recovered with 85% of the Amp^r His$^+$ exconjugant clones. Less than 1.5% loss of the RI host specificity genes occurred after one of these clones was propagated for 14 generations without antibiotic selective pressure.

Media, buffers, and antibiotics. Tryptone broth, L-broth, minimal media, diluents, and buffers have been described elsewhere (10, 19). Streptomycin sulfate was stored as 50 and 5 mg/ml solutions at −20 C and used at a final concentration of 200 μg/ml. Sulfathiazole, sodium salt, was stored at room temperatures as 20 mg/ml solutions and used at a final concentration of 200 μg/ml. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) was stored as a 1 mg/ml solution at −20 C and used at a final concentration of 50 or 100 μg/ml. Nalidixic acid (Winthrop Laboratories) was stored at room temperature in 0.1 N NaOH at a concentration of 20 mg/ml. Aqueous ampicillin solutions were prepared as needed.

Mutagenesis. Bacterial cultures were mutagenized with *N*-methyl-*N*-nitrosoguanidine (1).

Conjugational procedure. L-broth cultures of donor and recipient bacteria were mixed at a cell ratio of one and incubated in L-broth for 3 hr at 37 C and plated on selective medium after the appropriate dilutions were made. Selections were made with streptomycin, ampicillin, and sulfathiazole resistance, and counterselection was usually for amino acid requirements or nalidixic acid resistance.

Efficiency of plating. The appropriate dilutions of phage stocks of λ with different modifications were plated on bacterial cultures prepared by the procedure of Arber and Dussoix (2).

RESULTS

RI and RII host specificities. The *fi*$^-$ plasmid used here was characterized originally by Watanabe and Nishida (21). Arber and Morse (4) and Arber and Wauter-Willems (5) showed that the RII host specificity of this plasmid was different from the K, B, A, 15, and P1 host specificities. This plasmid was transferred to *E. coli* strains with K and B restriction and modification specificities (W3550 and HB129) as well as *E. coli* C (HB81).

The origin of the *fi*$^+$ plasmid used here was a clinical specimen of *E. coli*. Multiply drug-resistant clinical isolates were conjugated with an *E. coli* K-12 r_K^- m_K^+ Nal^r recipient. Selection was for the drug-resistant markers of both parents. The efficiency of plating of unmodified λ on each of the recombinant clones was determined. A total of 33 R factors were recovered from 214 clinical specimens. Six of the R factor-containing K-12 strains restricted unmodified λ. Five were determined to be *fi*$^-$, and they had the same host specificity as the Watanabe R factor. One R factor was *fi*$^+$, and its host specificity was different from the others.

This plasmid lost the ability to conjugate when it was established in the K-12 strain. The host specificity genes of this plasmid were recombined into a derepressed *fi*$^+$ plasmid obtained from R. Silver. The reconstructed plasmid with the RI restriction and modification genes was transferred to the HB129, W3350 and HB81 strains. It is assumed that these genes were physically integrated into the transferable plasmid. Table 1 is a composite of the efficiency of plating experiments for λ phage on the various strains. The results demonstrate that the RI and RII host specificities are mutually exclusive with each other and with the K, B, and P1 host specificities. The 15, A, and *S. typhimurium* host specificities are also different from the RI and RII host specificities (5; Slocum and Boyer, *unpublished data*). Strains containing both RI and RII host specificities restrict unmodified λ with an efficiency of plating equal to the product of the individual efficiencies of plating. The levels of restriction exerted by these RI and RII host specificities are similar to those found previously (6, 7), although the plasmids used in these studies were of a different origin.

Restriction mutants of the RI and RII host specificities. Restriction mutants of a number of host specificities (K, B, P1, 15, and A) are divided almost equally with respect to modification function, i.e., 50% are r^-m^+ and 50% are r^-m^- (15, 23). Since this mutant distribution indicates a unique relationship between the restriction endonuclease and modification methylase, i.e., sharing of one or more

TABLE 1. *Summary of efficiency of plating data*

Modification of λ phage	Efficiency of plating on:												
	C[a]	B	K	K-P1	C-RII	K-RII	B-RII	C-RI	K-RI	B-RI	C-RI-RII	K-RI-RII	B-RI-RII
C	1[b]	1×10^{-4}	1×10^{-4}	1×10^{-7}	5×10^{-3}	4×10^{-5}	7×10^{-5}	1×10^{-4}	5×10^{-7}	5×10^{-5}	4×10^{-7}	9×10^{-9}	2×10^{-8}
B	1	1	4×10^{-4}	7×10^{-7}	1×10^{-3}	2×10^{-6}	1×10^{-3}	9×10^{-5}	7×10^{-7}	1×10^{-4}	2×10^{-7}	5×10^{-8}	2×10^{-8}
K	1	1×10^{-4}	1	2×10^{-5}	9×10^{-3}	1×10^{-2}	2×10^{-5}	6×10^{-5}	2×10^{-4}	2×10^{-7}	5×10^{-7}	6×10^{-7}	8×10^{-8}
K-P1	1	1×10^{-4}	1	1	3×10^{-3}	7×10^{-3}	7×10^{-6}	8×10^{-5}	2×10^{-4}	6×10^{-7}			
C-RI	1	2×10^{-4}	9×10^{-4}	2×10^{-8}	3×10^{-2}	2×10^{-5}	3×10^{-5}	1	5×10^{-4}	5×10^{-4}	7×10^{-3}	5×10^{-5}	4×10^{-5}
C-RII	1	2×10^{-4}	8×10^{-4}	4×10^{-7}	1	9×10^{-4}	1×10^{-4}	2×10^{-4}	4×10^{-6}	1×10^{-6}	2×10^{-4}	4×10^{-6}	9×10^{-6}
B-RI	1	1	7×10^{-4}	1×10^{-8}	2×10^{-3}	4×10^{-6}	2×10^{-3}	1	6×10^{-4}	1	2×10^{-3}	1×10^{-5}	2×10^{-3}
K-RI	1	7×10^{-4}	1	3×10^{-5}	8×10^{-3}	2×10^{-2}	2×10^{-5}	1	1	9×10^{-4}	1×10^{-2}	3×10^{-3}	2×10^{-5}
B-RII	1	1	3×10^{-4}	2×10^{-7}	1	1×10^{-4}	1	8×10^{-5}	7×10^{-7}	1×10^{-4}	6×10^{-5}	7×10^{-7}	2×10^{-5}
K-RII	1	7×10^{-4}	1	2×10^{-5}	1	1	5×10^{-4}	9×10^{-5}	1×10^{-4}	1×10^{-7}	3×10^{-6}	1×10^{-4}	3×10^{-6}
C-RI-RII	1										1		5×10^{-3}
B-RI-RII	1										1	5×10^{-3}	1
K-RI-RII	1										1	1	1×10^{-3}

[a] Stocks of λ*vir* were prepared from each of the 13 strains listed above. The efficiency of plating of these stocks on the various host strains was determined as described in Materials and Methods.

[b] Designates restriction specificity of the host.

polypeptide subunits (10, 16), it was of interest to characterize the restriction mutants of the RI and RII host specificities.

E. coli strains carrying the fi^+ and fi^- plasmids were mutagenized by NTG, and restriction mutants were selected by the transduction procedure of Wood (23). The results are summarized in Table 2, and the striking feature of these restrictionless mutants is that in all cases they were r^-m^+. The failure to find the r^-m^- phenotype in the independently isolated mutants is significant at the 1% confidence level. Since the RII mutants were isolated in three different backgrounds, it is unlikely that any interaction with the products of the host restriction and modification alleles masked the phenotype. These results are in conflict with those reported by Bannister and Glover (7) who found some r^-m^- mutants of the RI and RII host specificities. The recovery of r^-m^- mutants in these cases might be explained in one of several ways: e.g., the RI and RII host specificity genes could have been deleted from the R factor; or the R factors themselves could have been lost by segregation; or the R factors reported here could have different host specificities.

Our results suggest that there is no gene product common to the RI restriction and modification enzymes nor the RII restriction and modification enzymes.

No complementation occurs between r_{II}^+ m_{II}^+/r_I^- m_I^+ nor r_I^+ m_I^+/r_{II}^- m_{II}^+ arrangements (Table 3). Complementation between r^-m^+ mutants of the same host specificity cannot be examined because of superinfection immunity (20).

DISCUSSION

Some fi^+ and fi^- plasmids (resistance trans-

TABLE 2. *Restriction mutants of RI and RII host specificities*[a]

Host cell specificity	Plasmid specificity	No. of colonies tested	No. of restrictionless mutants	Independent mutants	RI or RII phenotype
$r_K^+ m_K^+$	RI	91	57	9	r^-m^+
$r_K^+ m_K^+$	RII	134	70	14	r^-m^+
$r_0 m_0$	RII	95	35	7	r^-m^+
$r_B^+ m_B^+$	RII	219	80	14	r^-m^+

[a] The procedure of Wood (23) was used to select restriction mutants. All of the restriction-deficient mutants plated unmodified λ*vir* stocks with efficiencies of plating ranging from 0.5 to 1.0. Stocks of λ*vir* prepared from these mutant strains were completely modified, i.e., they plated with an efficiency of 1.0 on the parental strain.

TABLE 3. *Efficiency of plating of λ on strains carrying RI and RII plasmids*[a]

Modification of λ	Efficiency of plating on:			
	$r_K^+ m_K^+$[b] $r_{RI}^+ m_{RI}^+$ $r_{RII}^+ m_{RII}^+$	$r_K^+ m_K^+$ $r_{RI}^- m_{RI}^+$ $r_{RII}^+ m_{RII}^+$	$r_K^+ m_K^+$ $r_{RI}^+ m_{RI}^+$ $r_{RII}^- m_{RII}^+$	$r_K^+ m_K^+$ $r_{RI}^- m_{RI}^+$ $r_{RII}^- m_{RII}^+$
λ·K	3×10^{-7}	2×10^{-3}	5×10^{-5}	1.0
λ·K, RI	1×10^{-2}	5×10^{-3}	1.0	1 0
λ·K, RII	7×10^{-5}	1.0	8×10^{-5}	1.0
λ·K, RI, RII	1.0	1.0	1.0	1.0

[a] Strains containing both plasmids were constructed as described in Materials and Methods.

[b] Designates phenotypes of individual alleles present in organisms.

fer factors) carry genes for host-controlled restriction and modification enzymes (6, 7, 21, 22). The host specificities of the plasmids described here are mutually exclusive, and on the basis of efficiency of plating data these RI and RII host specificities appear to be the same as those described previously (6, 7). This brings the number of different host specificities known in *E. coli* to seven (5; Slocum and Boyer, *unpublished data*).

The absence of modification mutants in the RI and RII restriction mutant populations described here suggested to us that the genetic control of the RI and RII restriction endonucleases and modification methylases might be different than the genetic control of the K and B host specificities. The absence of complementation between the RI and RII host specificities (in contrast to B and K) also suggests little similarity between the RI and RII restriction and modification enzymes. We conclude from our observations that in both the RI and RII host specificities no polypeptide subunit is common to the restriction endonuclease and modification methylase. Therefore, in these cases, the restriction endonucleases could be controlled by one gene and the modification methylases by another gene. In the next two papers of this series we will present evidence to support the above conclusions (Yoshimori, Roulland-Dussoix, Aldridge, and Boyer, *unpublished;* Yoshimori, Roulland-Dussoix, Goodman, and Boyer, *unpublished*). This proposal is in contrast to the genetic control of the K and B host specificities where three genes are known to control the restriction endonucleases and modification methylases (10, 16).

Since Bannister and Glover (7) reported the recovery of r^-m^+ and r^-m^- mutants of the RI and RII host specificities, it is apparent that in some cases these data alone are not adequate for making decisions about the subunit structures of the restriction endonucleases and modification methylases, especially when the host specificities are genetically controlled by plasmids that are known to incur deletion losses (12).

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