

HOST SPECIFICITY OF DNA IN HAEMOPHILUS INFLUENZAE:RESTRICTION AND MODIFICATION IN STRAIN R<sub>d</sub>

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## SUMMARY

Wild type strains of Haemophilus influenzae R<sub>d</sub> consist of two phenotypic classes of bacteria which differ in their abilities to restrict and modify phage HP1. Each of these classes  $r_{D}^{-} m_{D}^{-}$  and  $r_{D}^{+} m_{D}^{+}$  is unstable and segregates several percent of bacteria of the alternative phenotype. In addition stable  $r_{D}^{-} m_{D}^{-}$  bacteria occur spontaneously in  $r_{D}^{+} m_{D}^{+}$  cultures and an  $r_{D}^{-} m_{D}^{-}$  mutant was isolated after mutagenesis of an  $r_{D}^{+} m_{D}^{+}$  strain.

## INTRODUCTION

Each of the serological types of H. influenzae R<sub>a</sub>, R<sub>b</sub>, R<sub>d</sub>, R<sub>e</sub> and R<sub>f</sub> carries at least one restriction and modification system (3) which can be detected using the Haemophilus phages HP1, HP1c1 and S2. A restriction endonuclease isolated from strain R<sub>d</sub> degrades foreign DNA (including T7, P22 and B. subtilis DNA) but has no detectable activity against homologous DNA (5). This enzyme recognises a sequence of six nucleotide pairs with characteristic twofold rotational symmetry (2). In this report we describe some preliminary observations on the genetic analysis of the determinants for restriction and modification in H. influenzae R<sub>d</sub>.

## RESULTS AND DISCUSSION

The wild type strain of H. influenzae R<sub>d</sub> used initially in

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these experiments was obtained from R. M. Herriott and another strain Rd200 was obtained from J. Stuy. It was assumed that these strains were phenotypically  $r^+ m^+$  and our object was to isolate restriction-deficient mutants following N-methyl-N-nitro-N-nitrosoguanidine (NTG) mutagenesis. However, instead of isolating restriction-deficient mutants we obtained mutants which restricted Haemophilus phages previously grown on the wild type strains (Table 1). Single cycle phage infection experiments

TABLE 1

The efficiency of plating of phage HP1c1  
on strains of H. influenzae Rd

Phage <sup>4</sup>	Host strain		
	Rd (Herriott) <sup>3</sup>	Rd (Stuy) <sup>2</sup>	Rd 123 <sup>1</sup>
HP1c1.Rd (Herriott)	1.0	1.0	$2 \times 10^{-2}$
HP1c1.Rd (Stuy)	0.8	1.0	$2 \times 10^{-2}$
HP1c1.Rd 123	1.0	1.0	1.0
HP1c1.Rd 123.Rd (Stuy)	0.8	1.0	$2 \times 10^{-2}$

1. Rd 123 an  $r_D^+ m_D^+$  mutant isolated from Rd (Stuy).
2. Rd (Stuy) strain Rd 200 (ref. 6); phenotype  $r_D^- m_D^-$ .
3. Rd (Herriott) strain Rd from R. M. Herriott; phenotype  $r_D^- m_D^-$ .
4. Phages HP1 and S2 gave similar results.

confirmed that strain Rd200 displays an  $r^- m^-$  phenotype and the mutants e.g. Rd 123 display an  $r^+ m^+$  phenotype (Table 2). These results show that  $r^+ m^+$  derivatives of strain Rd plaque phage HP1c1.Rd200 with an efficiency of  $2 \times 10^{-2}$  and that virtually all of the phage is host-modified after one cycle of growth. However, the fraction of progeny phage able to plaque on Rd 123  $r^+ m^+$  following infection of Rd 200  $r^- m^-$  with phage HP1c1.Rd 123 was unexpectedly high (6%). This system of restriction and

TABLE 2

Growth of HP1c1.0 and HP1c1.D in H. influenzaeRd  $r_D^+$   $m_D^+$  and Rd  $r_D^-$   $m_D^-$  strains\*

	Growth of HP1c1.0 in		Growth of HP1c1.D in	
	<u>Rd</u> $r_D^-$ $m_D^-$	<u>Rd</u> $r_D^+$ $m_D^+$	<u>Rd</u> $r_D^-$ $m_D^-$	<u>Rd</u> $r_D^+$ $m_D^+$
Number of bacteria per ml.	$1.5 \times 10^8$	$1.1 \times 10^8$	$1.5 \times 10^8$	$1.3 \times 10^8$
Number of phage per ml.	$5.2 \times 10^6$	$5.2 \times 10^6$	$7.7 \times 10^6$	$7.7 \times 10^6$
Multiplicity of infection	0.04	0.04	0.05	0.05
Adsorption	83%	77%	50%	48%
Number of infective centres/ml. on:	<u>Rd</u> $r_D^-$ $m_D^-$	$1.5 \times 10^6$	$1 \times 10^5$	$4 \times 10^6$
	<u>Rd</u> $r_D^+$ $m_D^+$	$5.5 \times 10^4$	$4 \times 10^4$	$2 \times 10^6$
Transmission coefficient	0.4	0.02	1.0	1.0
Progeny phage per ml. on:	<u>Rd</u> $r_D^-$ $m_D^-$	$1.0 \times 10^8$	$6 \times 10^6$	$4.3 \times 10^8$
	<u>Rd</u> $r_D^+$ $m_D^+$	$5 \times 10^5$	$3 \times 10^6$	$2.5 \times 10^7$
Average burst size	66	75	100	60
Fraction of HP1c1.D among progeny	0.5%	50%	6%	100%

\* Rd  $r_D^+$   $m_D^+$  was Rd 123 an  $r_D^+$   $m_D^+$  mutant isolated from Rd (Stuy)<sub>3</sub>; Rd  $r_D^-$   $m_D^-$  was strain Rd 200 from J. Stuy.

modification is designated D in conformity with the nomenclature of Arber and Linn (1969). The phenotype of Rd 200 is thus  $r_D^- m_D^-$  and the mutants  $r_D^+ m_D^+$ . Phage grown on  $r_D^- m_D^-$  strains is designated HP1c1.0 and when grown on  $r_D^+ m_D^+$  is designated HP1c1.D.

The number of  $r_D^+ m_D^+$  mutants (15-20% of all tested colonies) isolated from mutagenised cultures of Rd 200 was unexpectedly high. In addition, a similar high proportion of mutants ( $r_D^- m_D^-$ ) was observed following treatment of strain Rd 123  $r_D^+ m_D^+$  with NTG. It became obvious that mutagenic treatment was unnecessary and that strains with the alternative host specificity phenotype could be obtained spontaneously with high frequency.

To obtain information about the spontaneous instability of the restriction and modification phenotypes of these strains several hundred colonies of Rd 200 and Rd 123 were screened for their host specificity phenotypes with phage HP1c1. Cultures of Rd 200 yield about 5 to 10% of colonies with an  $r_D^+ m_D^+$  phenotype while cultures of Rd 123 yield about 2-6% of colonies which are  $r_D^- m_D^-$ . Further,  $r_D^+ m_D^+$  strains obtained from  $r_D^- m_D^-$  cultures and  $r_D^- m_D^-$  strains obtained from  $r_D^+ m_D^+$  cultures display the same degree of instability of genetic expression.

These results explain the unexpectedly high fraction of phage, obtained following infection of Rd 200  $r_D^- m_D^-$  with HP1c1.D, which was able to plaque on Rd 123  $r_D^+ m_D^+$ . This fraction (6%) corresponds to the fraction of  $r_D^+ m_D^+$  bacteria present in an  $r_D^- m_D^-$  culture. On the other hand, since  $r_D^- m_D^-$  cultures contain a high frequency of  $r_D^+ m_D^+$  bacteria, phage stocks, prepared on such cultures, will contain a high frequency of host modified phage particles and the e.o.p. of such a stock on  $r_D^+ m_D^+$  indicator bacteria will reflect their presence. Thus it might be anticipated that HP1c1 prepared on a quite different strain of H. influenzae would plaque on Rd 123  $r_D^+ m_D^+$  indicator bacteria with a much lower frequency and this has been confirmed (3). The e.o.p. on Rd 123  $r_D^+ m_D^+$  obtained for phage HP1c1 previously grown on H. influenzae Rd strains is thus a measure of the fraction of  $r_D^+ m_D^+$  bacteria present in the culture. In Table 3 we list various H. influenzae Rd strains obtained from different laboratories together with their restriction and modification phenotypes and an estimate of the percent of the alternative phenotype found. It is clear that some Rd strains are essentially  $r_D^+ m_D^+$ , others  $r_D^- m_D^-$  and some are mixtures in almost equal numbers of both phenotypes. It is interesting to note that the strain

TABLE 3

The restriction and modification phenotypes  
of H. influenzae Rd strains obtained from different sources

Strain and source	Restriction. e.o.p. phage HP1c.0	Modification e.o.p. phage HP1c.1.strain on:		Phenotype	% minority phenotype	
		$r_D^-$ $m_D^-$	$r_D^+$ $m_D^+$		$r_D^-$ $m_D^-$	$r_D^+$ $m_D^+$
Rd 200 (Stuy)	1.0	1.0	$2 \times 10^{-2}$	$r_D^- m_D^-$	5-10%	$r_D^+$ $m_D^+$
Rd 123 (isolated from Rd 200)	$2 \times 10^{-2}$	1.0	1.0	$r_D^+$ $m_D^+$	5%	$r_D^- m_D^-$
Rd (Herriott)	0.8	1.0	$3 \times 10^{-2}$	$r_D^- m_D^-$	15%	$r_D^+$ $m_D^+$
Rd (Setlow)	$2 \times 10^{-2}$	1.0	1.0	$r_D^+$ $m_D^+$	1%	$r_D^- m_D^-$
Rd (Smith)	$1 \times 10^{-1}$	1.0	0.5	$r_D^+$ $m_D^+$	<sup>4</sup> 50%	$r_D^+$ $m_D^+$
Rd (Leidy)	$2 \times 10^{-2}$	1.0	1.0	$r_D^+$ $m_D^+$	50%	$r_D^- m_D^-$
<sup>1</sup> Rd 221 (Stuy)	-	1.0	$5 \times 10^{-2}$	$r_D^- m_D^-$	5%	$r_D^- m_D^-$
<sup>2</sup> Rd S-19 (Goodgal)	-	1.0	$5 \times 10^{-2}$	$r_D^- m_D^-$	-	-
<sup>3</sup> RdC Rd (Herriott)	-	1.0	$3 \times 10^{-2}$	$r_D^- m_D^-$	-	-

1. Rd 221 (Stuy) is lysogenic for phage HP1
2. Rd S-19 (Goodgal) is lysogenic for phage S2
3. RdC Rd (Herriott) is lysogenic for phage HP1c.1.
4. Isolated clones of  $r_D^+$   $m_D^+$  behave like Rd 123; isolated clones of  $r_D^- m_D^-$  behave like Rd 200.
5. About 90% of colonies in the original isolate were resistant to HP1c.1 and S2; sensitive colonies contain 95%  $r_D^+$   $m_D^+$  and 5%  $r_D^- m_D^-$  bacteria.

obtained from Dr. H. O. Smith from which a restriction endonuclease has been isolated (5) was approximately 50%  $r_D^+$   $m_D^+$  and 50%  $r_D^- m_D^-$ .

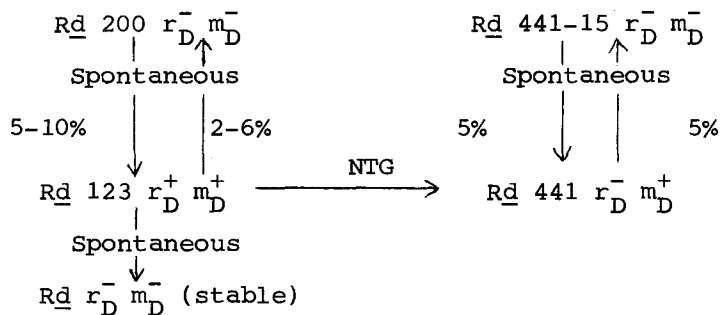
Host-controlled restriction and modification in H. influenzae Rd was first described several years ago (4). These authors noted that bacteriophage grown on certain UV sensitive mutants was restricted by wild type Rd strains, whereas phage grown on the wild type strain was not restricted by these mutants. The

same authors also noticed the very high frequency of host-modification mutants isolated following the treatment of wild type Rd with NTG.

Following treatment of Rd 123 with NTG we isolated a mutant, Rd 441, with the phenotype  $r_D^- m_D^+$  in addition to the usual high frequency of  $r_D^- m_D^-$  derivatives. It seemed likely from previous experience (3) that this mutant carried a mutation in one of the host specificity genes and it was of interest therefore to examine the stability of this new phenotype. Clonal analysis, as carried out previously, revealed that cultures of Rd 441  $r_D^- m_D^+$  yielded about 5% of colonies with the  $r_D^- m_D^-$  phenotype. One of these  $r_D^- m_D^-$  derivatives (Rd 441-15) was equally unstable and on cloning yielded about 5% of colonies which, like the parent strain Rd 441, were  $r_D^- m_D^+$ . No  $r_D^+ m_D^+$  colonies were obtained among several hundred colonies of Rd 441  $r_D^- m_D^+$  and Rd 441-15  $r_D^- m_D^-$  screened. It is most probable therefore that strain Rd 441  $r_D^- m_D^+$  carries a mutant hsr gene (1) and that this mutant phenotype is subject to the same instability of expression as the wild type  $r_D^+ m_D^+$  strains.

During clonal analysis of Rd 200 and Rd 123 another class of mutants was obtained. These mutants were isolated only from Rd 123. They do not restrict phage HP1c1 and phage stocks prepared on them plaque on Rd 123 with an efficiency of  $1 \times 10^{-3}$ . This is taken to mean that such phage stocks contain less than 1 in 1000 phage particles carrying the D-type modification and therefore that this class of  $r_D^- m_D^-$  mutants is stable. Analysis of several hundred colonies isolated from such a mutant failed to reveal any which were  $r_D^+ m_D^+$ .

The instability of the restriction and modification phenotypes of these derivatives of H. influenzae Rd can be summarised as follows:



The fact that  $r_D^+ m_D^+$  cultures contain a high frequency of  $r_D^- m_D^-$  cells as well as  $r_D^- m_D^-$  (stable) bacteria makes it impossible to identify  $r_D^- m_D^-$  mutants that might arise as a result of mutation in a host specificity gene common to restriction and modification (1).

It has not escaped our attention that these results are consistent with the notion that in *H. influenzae* Rd host specificity genes may be carried on a plasmid and experiments are in progress to test this hypothesis.

We do not know whether the restriction studied here in strain Rd is due to the restriction endonuclease previously described (4). The instability of the restriction and modification phenotypes of *H. influenzae* Rd strains, and in particular our observation that strains of Rd provided by H. O. Smith contained 50% of  $r_D^- m_D^-$  bacteria renders this important point obscure. What is required is a comparison of the restriction endonuclease activity of Rd  $r_D^+ m_D^+$  and Rd  $r_D^- m_D^-$  (stable) strains.

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