

# RESTRICTION OF DNA IN HAEMOPHILUS INFLUENZAE

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## ABSTRACT

Each of the main serotypes of Haemophilus influenzae exerts type-specific restriction on DNA which can be detected using the Haemophilus phages HP1 and S2. Each strain restricts the growth of these phages previously grown on other strains.

Three strains have been examined in detail, Ra, Rb and Rd. A summary of these experiments will be presented and discussed in the light of recent studies on restriction endonucleases isolated from H. influenzae.

## 1. INTRODUCTION

The reason for our interest in Haemophilus influenzae stems from a long-term interest in the host-specificity of bacterial DNA.

It has been known for a long time that many species of bacteria possess a mechanism for screening invading DNA molecules. This mechanism is independent of the source of the DNA and rejects those that are detected as 'foreign', that is, of a different host-specificity from that of the recipient strain. The

rejection mechanism involves primarily the action of specific deoxyriboendonucleases which cleave the DNA molecules at certain specific sites. They are able to do this as a consequence of recognising the base sequence at these sites. Since these sequences are relatively short they occur in the DNA of the restricting strain itself which must therefore be possessed of a mechanism preventing endonuclease attack upon its own DNA. This second mechanism is the process of DNA modification. Modification frequently consists of the alteration by methylation of a base or bases in these short sequences so that the endonuclease is no longer able to bind to the substrate.

The first of these sequences to be analysed was the one recognised by an endonuclease from Haemophilus influenzae. This enzyme has been isolated and purified from Haemophilus influenzae Rd. It degraded foreign DNA but not Haemophilus influenzae DNA, and the sequence of bases around the cleavage point has been determined. (Smith & Wilcox, 1970; Kelly & Smith, 1970).

By this time we had considerable knowledge of the genetic control of restriction and modification in Escherichia coli K12 and related strains. It was possible to build up a picture of the endonucleases and methylases involved in these host-specificity systems. There is good evidence that the endonuclease consists of three different sub-units each coded for by a different gene.

These genes have been given the symbols hss, hsr and hsm, and it is an extension of the three gene hypothesis that the hss coded sub-unit plays an essential role in site recognition. The methylase is controlled by two genes hss and hsm and thus contains only two types of sub-unit. There is evidence that some plasmid controlled host-specificity systems might be somewhat

simpler - controlled only by two genes. (See reviews by Arber & Linn, 1969; Boyer, 1971; Meselson, Yuan & Heywood, 1972). To bridge the gap between, on the one hand, the genetic studies in E. coli and, on the other hand, the sequence studies in Haemophilus influenzae, we began a study of the genetics of restriction and modification of DNA in Haemophilus.

## 2. RESTRICTION AND MODIFICATION OF DNA IN HAEMOPHILUS INFLUENZAE RD.

The Haemophilus influenzae strain Rd is the one most widely used in transformation studies and was the strain from which the restriction endonuclease had been isolated. It was a basic assumption at the start of these experiments that the endonuclease R isolated by Smith & Wilcox (1970) was a component of a restriction and modification system. Our objective was to isolate mutants deficient in endonuclease R and/or deficient in the modification that was assumed to be part of this host-specificity system. Fundamentally these experiments failed - in that we did not isolate mutants deficient in endonuclease R, but they did enable us to define, at least in biological terms, a new restriction enzyme and a new modification enzyme.

In the first series of experiments strain Rd was lysogenised with the Haemophilus phage HPl; the lysogenic culture was treated with the mutagen N-methyl-N-nitroso-N'-nitroguanidine (Glover & Piekarowicz, 1972) and plated out on Brain-Heart Infusion (BHI) agar to form colonies. The colonies were then replica-plated on to lawns of the sensitive indicator strain Rd. It was anticipated that lysogenic colonies of the wild-type strain Rd would produce a zone of lysis on the indicator plates caused by the

spontaneous release of phage HP1. Lysogenic colonies of strain Rd in which the modification process was deficient were expected to yield HP1 phage which would be restricted by the indicator strain and thus these colonies would produce a reduced zone of lysis or no lysis at all (see Fig. 1).

It proved relatively easy to obtain colonies which failed to lyse the indicator strain but the reason for this failure was most frequently due to the presence of defective phage (Stuy, 1969) rather than to restriction.

In the second series of experiments exposure of phage HP1 to the mutagen was avoided. Cultures grown from colonies of a mutagenised suspension of Haemophilus influenzae Rd were infected with a predetermined number of HP1 phages and the phage was allowed to grow. After growth a carefully controlled volume of the suspension was plated on the indicator strain Rd. It was anticipated that phage from wild-type Rd cultures would produce plaques on the indicator strain but that phage from modification deficient mutants of Rd would not, or would produce very few plaques (see Fig. 2).

As before it proved rather easy to obtain mutants which appeared, by this test, to be modification deficient. However, further investigation showed that the reason why phage from these mutants produced few plaques on the indicator strain was because there was very little phage in the lysates and not because the phage was restricted by the indicator bacteria.

We were able to show that these mutants, instead of being modification deficient as we had supposed, were able to restrict the infecting phage and thus very few of the infected bacteria produced progeny phage. Since initially the strain had

been assumed to be  $r^+ m^+$  (restriction and modification proficient), we could suppose that the mutants were  $r^{+'} m^{+'}$  where prime (') indicates an altered host-specificity. These mutants would have been of great interest since persistent attempts to isolate mutants with altered host-specificity in Escherichia coli had previously failed (Glover, unpublished). However, it was soon discovered that the starting strain was  $r^- m^-$  and that the so-called mutants were  $r^+ m^+$  and that both of these phenotypes are unstable (Fig. 3).

The genetic basis of this instability remains obscure. The most obvious explanation of these observations is that the instability of this system is determined by the alternating locations of a plasmid which can be irreversibly lost. However, treatments which effectively cure Escherichia coli of plasmids failed to have consistent effects on this phenomenon. Attempts to transfer the plasmid from  $r^+ m^+$  strains to  $r^- m^-$  'stable' strains in mixed culture were unsuccessful, although some initial experiments appeared to demonstrate that the plasmid was transferable. These initial experiments were explained when it was found that the 'stable'  $r^- m^-$  strains do in fact segregate  $r^+ m^+$  strains rarely - with a frequency 10 to 100 times lower than unstable  $r^- m^-$  strains.

One other point of interest is that another characteristic of Haemophilus influenzae Rd shows almost identical instability, that is, resistance to phage HPl (Stuy, 1968). However, these two instabilities, host-specificity and phage resistance, are genetically independent and clearly controlled by different elements.

That the restriction endonuclease, which must be a component of the  $r_D^+ m_D^+$  host-specificity system just described, is

not the restriction endonuclease described by Smith & Wilcox (1970) became apparent when it was shown that our  $r_D^-$  mutants still contained endo. R (Paul Roy, personal communication). Thus Haemophilus influenzae Rd contains at least two restriction endonucleases.

Results similar in all important respects to these have been obtained by Goodgal (Gromkova & Goodgal, 1972; and personal communication), using Haemophilus phage S2 and the role that restriction endonucleases play in heterospecific transformation has also been investigated by Goodgal (Goodgal - This Symposium). Obviously, the restriction exercised by  $r_D^+ m_D^+$  strains against the DNA of phage from  $r_D^- m_D^-$  strains presents a potential problem in any genetic transformation experiments carried out between Rd strains which differ in host-specificity.

### 3. HOST-SPECIFICITY OF DNA IN HAEMOPHILUS INFLUENZAE RA

Haemophilus influenzae Ra carries two genetically distinct host-specificity systems A1 and A2, each of which is able to restrict Haemophilus phage HPl, and each of which confers a specific modification on phage grown in strain Ra. Among restriction-deficient mutants isolated from strain Ra, seven of the eight possible phenotypes for these two systems were obtained after either one or two mutational steps. The eighth phenotype was not specifically screened for (Piekarowicz & Glover, 1972). Both of these host-specificities, A1 and A2, are genetically stable in their expression (see Table I).

4. HOST-SPECIFICITY OF DNA IN HAEMOPHILUS INFLUENZAE RB

The host-specificity of Haemophilus influenzae Rb has been investigated following the methods described by Piekarowicz and Glover (1972). Phage HPlc1 grown on other Haemophilus influenzae strains forms plaques on strain Rb with an efficiency of  $3 \times 10^{-4}$  (Table 1). Restriction-deficient mutants have been isolated after mutagenesis and scored for restriction and modification, using phage HPlc1. The mutants display a wide range of restriction-deficient phenotypes on which phage HPlc1.0 forms plaques with efficiencies that vary from 1.0 to  $1 \times 10^{-3}$ . That restriction-deficient  $r_B^-$  mutants can be isolated as a result of a single mutagenic treatment indicates that strain Rb possesses only a single host-specificity system, in contrast to strain Ra (see above) which has two such systems A1 and A2 (Piekarowicz & Glover, 1972). The modification phenotypes of the mutants so far examined do not display the same variety as the restriction phenotypes. The mutants are either  $m_B^+$  or  $m_B^-$ . The  $r_B^+ m_B^+$  host-specificity system defined here does not display the instability characteristic of strain Rd.

5. HOST-SPECIFICITY OF DNA IN HAEMOPHILUS INFLUENZAE RC

Phages HPl and S2 grown on other strains of Haemophilus influenzae does not form plaques on strain Rc. The reason for this failure to form plaques has not been investigated. If strain Rc is resistant to phage HPl then sensitive mutants are rare since several thousand colonies have been screened after mutagenesis without yielding a sensitive survivor.

6. HOST-SPECIFICITY OF DNA IN HAEMOPHILUS  
INFLUENZAE RE AND RF

Phage HPl<sub>cl</sub> grown on other strains of Haemophilus influenzae forms plaques on strains Re and Rf with a very low efficiency (approximately  $1 \times 10^{-9}$ ). On the other hand, phage HPl previously grown on strain Re or Rf forms plaques on Re and Rf with an efficiency of 1.0 or very close to 1.0. These strains therefore possess a host-specificity quite distinct from the other Haemophilus influenzae strains and it is probable that Re and Rf do not differ from one another in their DNA host-specificities (see Table 1).

The extremely low efficiency of plating of phage HPl.0 on strains Re and Rf very probably indicates the presence of two and possibly even three independent restriction endonucleases.

It has been known for a long time that strain Rd is the strain most readily transformable by isolated DNA. Other strains have been reported to be poorly transformable or not transformable at all. Whether the reason for this poor transformability is due to problems of competence or whether it is due to restriction of transforming DNA is under investigation.

In summary, these experiments define at least five and possibly seven different restriction endonucleases potentially able to degrade heterologous DNA molecules.

TABLE I  
EFFICIENCY OF PLATING OF PHAGE HP1 ON STRAINS OF  
HAEMOPHILUS INFLUENZAE

<u>Phage grown on</u> <u>H. influenzae</u>	Host strains *						
	<u>0</u>	<u>Ra</u>	<u>Rb</u>	<u>Rc</u>	<u>Rd</u>	<u>Re</u>	<u>Rf</u>
HP1 . <u>0</u>	1.0	$5 \times 10^{-6}$	$3 \times 10^{-4}$	n.p.	$2 \times 10^{-2}$	$1 \times 10^{-9}$	$1 \times 10^{-9}$
HP1 . <u>Ra</u>	0.5	1.0	$3 \times 10^{-4}$	n.p.	$2.5 \times 10^{-4}$	$\leq 10^{-9}$	$\leq 10^{-9}$
HP1 . <u>Rb</u>	1.0	$5 \times 10^{-6}$	1.0	n.p.	$1 \times 10^{-3}$	$\leq 10^{-9}$	$\leq 10^{-9}$
HP1 . <u>Rd</u>	1.0	$5 \times 10^{-6}$	$3 \times 10^{-4}$	n.p.	1.0	$\leq 10^{-9}$	$\leq 10^{-9}$
HP1 . <u>Re</u>	1.0	$5 \times 10^{-6}$	$3 \times 10^{-4}$	n.p.	$1 \times 10^{-3}$	1.0	0.5
HP1 . <u>Rf</u>	1.0	$5 \times 10^{-6}$	$3 \times 10^{-4}$	n.p.	$1 \times 10^{-3}$	0.5	1.0

\* The strain designated 0 is an  $r_D^- m_D^-$  derivative of Haemophilus influenzae Rd; the strain designated Rd is an  $r_D^+ m_D^+$  derivative of Haemophilus influenzae Rd; n.p. indicates that no plaques were obtained from the highest concentration of phage lysate tested ( $1 \times 10^{10}$  particles/ml).

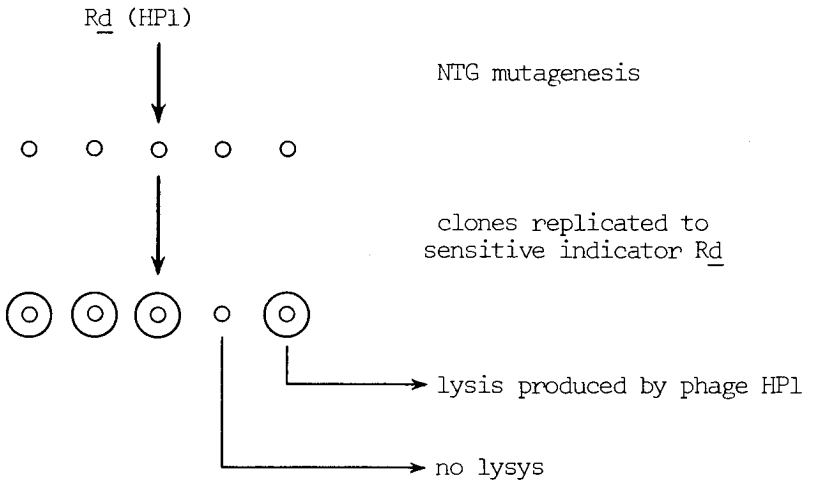


Fig. 1 - Experimental procedure used in initial experiments designed to isolate modification deficient mutants of Haemophilus influenzae Rd. For further explanation see text.

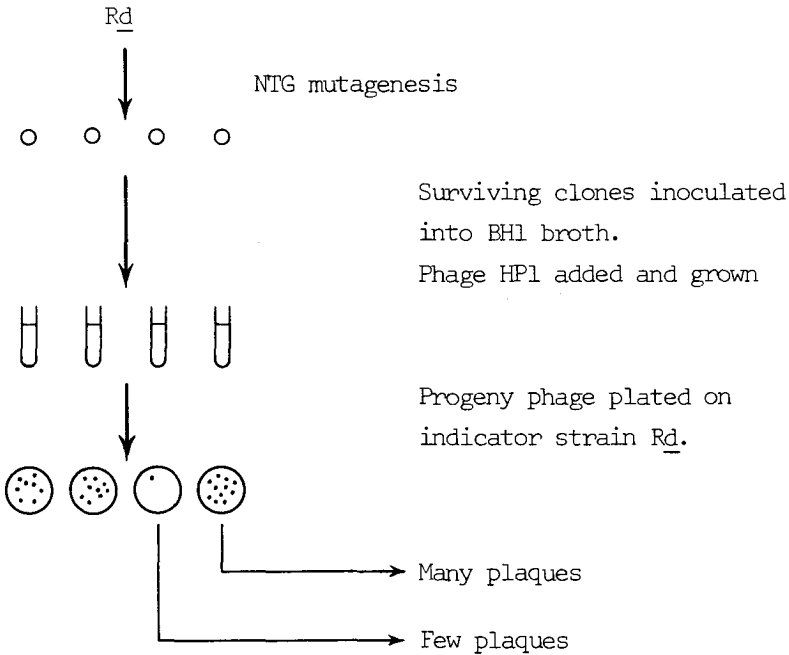


Fig. 2 - Experimental procedure used in further experiments designed to isolate modification deficient mutants of Haemophilus influenzae Rd. For further explanation see text.

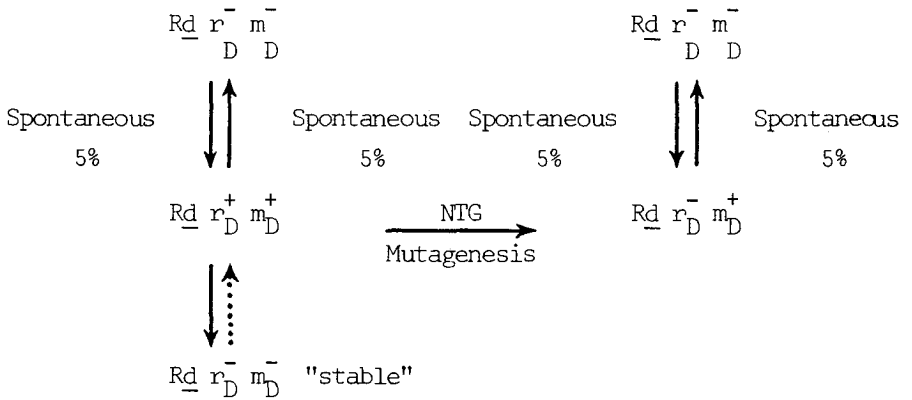


Fig. 3 - Instability of expression of host-specificity type D in Haemophilus influenzae Rd (after Glover & Piekarowicz, 1972).

## 7. REFERENCES

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