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Restriction Enzymes Do Not Play a Significant Role in *Haemophilus* Homospecific or Heterospecific Transformation

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Competent *Haemophilus influenzae* Rd recipients, either as phage HP1 restricting (r^+) or nonrestricting (r^-) nonlysogens or defective lysogens, were exposed to deoxyribonucleic acids from various wild-type phage HP1 lysogenic *H. influenzae* serotype strains (non-encapsulated derivatives of serotypes *a, b, c, d,* and *e*), to DNA from lysogenic *Haemophilus parahaemolyticus*, and to DNA from modified and nonmodified phage HP1. Transformation of antibiotic resistance markers and of prophage markers in homospecific crosses was observed to be unaffected by the recipient restriction phenotype, whereas the transfection response was much reduced in r^+ recipients. Heterospecific transformation of prophage markers was reduced by only 80 to 90%, whereas antibiotic resistance marker transformation was 1,000 to 10,000 times lower. Heterospecific transfection was at least 100 times lower than homospecific transfection in both r^+ and r^- recipients. The general conclusion is that neither class I nor class II restriction enzymes affect significantly the transformation efficiency in homospecific and heterospecific crosses. The efficiency of heterospecific transformation may depend mainly on the deoxyribonucleic acid homology in the genetic marker region.

A general feature of heterospecific bacterial genetic transformation is the low efficiency of marker transfer from donor to heterospecific recipient (for a general review, see reference 1; for *Haemophilus*, see references 3, 16, 18, 27). Exceptions have been observed, however (14). The extent of the reduction is particular for a given marker. Explanations of this phenomenon can be based on either (i) reduced uptake of the heterospecific deoxyribonucleic acid (DNA), (ii) the presence of so-called restriction enzymes (32) in the recipient that destroy the genetic integrity of adsorbed nonmodified DNA either before or after integration, or (iii) the lack of homology between donor and recipient DNA. Although competent *Haemophilus influenzae* Rd cells show a reduced uptake of unrelated foreign DNA (15, 28), the uptake of heterologous *Haemophilus parainfluenzae* DNA appears to be normal (18, 34). Moreover, the molecular fate of this DNA is comparable to that of homologous DNA after uptake (3, 18, 34, 36). This appears to rule out a major role of restriction enzymes at the preintegration stage. It has also been shown that *H. parainfluenzae* DNA is integrated into the *H. influenzae* genome (18, 34). This integration causes the death of a considerable fraction of the recipient cells through induction of the defective prophage (29) that is present in the Rd strain used most widely (4, 33). Since a presumably cured strain (2, 4) is insensitive to this lethal effect and since heterospecific transformation is also normally inefficient in this strain (29), the prophage induction phenomenon is not believed to be the explanation for the reduced heterospecific transformation efficiency. The lowered marker transfer is thus probably caused by events that follow integration (for a thorough discussion, see reference 17). Restriction enzymes, as well as the degree of DNA homology in the region of the genetic marker, might play important roles in the postintegration events.

To establish more clearly the roles of the degree of DNA homology and the presence of restriction enzymes on heterospecific transformation, I performed crosses in which homologous, as well as heterologous, donor DNA markers, present in one species of DNA, were transferred to a pair of isogenic recipients that were either able (r^+) or unable (r^-) to restrict some of these markers. This was done by using the *H. influenzae* HP1 prophage transformation system (37). Isogenic pairs of recipients were either phage HP1 restricting (r^+) or nonrestricting (r^-) strains (6), or they were defectively lysogenic derivatives. Donor DNA was either extracted from modifying ($m^+ r^+$) homologous strains of serotype *a, b, c, d,* and *e* (23), from nonmodifying ($m^- r^-$) strain Rd, or from

heterologous modifying (?) *H. parahaemolyticus*. Reports on the phage HP1 restriction properties of some of the various strains of *H. influenzae* have been published by others (6, 9, 20, 21, 22) and by Stuy (submitted for publication). At least some of the genes involved in modification and restriction have been located (9). My results show that phage restricting (r^+) *H. influenzae* Rd recipients were transfected much less efficiently than r^- ones when exposed to DNA extracted from nonmodified phage, from a nonmodifying, lysogenic strain Rd mutant (6), or from lysogenic strains Ra, Rb, Rc, and Re. DNA from a modifying lysogenic Rd strain transfected r^- and r^+ Rd recipients equally well. Transfection, at least in the Rd strains studied, is thus sensitive to restriction. This confirms earlier reports on *H. influenzae* (7, 8, 30) and on *Bacillus subtilis* (39). However, when isogenic r^- and r^+ defectively lysogenic strain Rd recipients were exposed to the homologous DNAs, prophage transformation, as well as antibiotic resistance transformation, was only slightly reduced, at most. When heterologous *H. parahaemolyticus* donor DNA was used, on the other hand, prophage transformation was reduced by only 80 to 90%, whereas two antibiotic resistance marker transformation efficiencies were several orders of magnitude lower. Heterospecific prophage transfection was at least 100 times lower in both r^- and r^+ recipients. The conclusion drawn from these data is that restriction enzymes play no major role in homospecific or heterospecific transformation.

MATERIALS AND METHODS

Strains. Table 1 lists all original cultures used, which are part of a large collection of hospital isolates sent to me by a number of investigators (Stuy, submitted for publication). The Rd strains used as recipients were derived from the original isolate "Garfeseous" described by Alexander and Leidy (1). Phage HP1 (11) was a gift from C. Rupert.

Transformation of bacterial antibiotic resistance markers (38) and of prophage markers (37) has been described. All assays of biological activities were done with limiting DNA concentrations. Transfection was performed by exposing competent cells for 30 min at 30°C to transfecting DNA at limiting concentrations. Suspensions were then diluted and plated in soft agar on hard agar. Indicator cells were added where necessary. Further incubation was at 38°C.

Preparation of phage HP1 lysogens. Enough phage was plated on the various cultures to give about 100 plaques. Lysogens were obtained from the centers of these plaques. They were genetically purified until they were observed to be stable phage producers. Strains e and f did not give plaques under these conditions, and these cultures were therefore tested with large numbers of phage. After overnight

TABLE 1. *Origin and designation of Haemophilus strains*

Strain	Original designation	Sensitive to phage HP1	Source
H. influenzae			
657 Sa	SM4	Yes	Leanne Heath, Center for Disease Control, Atlanta
686 Ra[a]	Smith	Yes	Grace Leidy, New York
688 Ra[a]	Cascia	Yes	Grace Leidy
701 RbI[a]	Santo	Yes	Grace Leidy
1057 SbI	74-64184	No	Linda Kirven, Center for Disease Control, Atlanta
1076 RbI[a]	Schneider	Yes	Grace Leidy
1090 SbII	B5-005	No	Carol Lewis, Tallahassee
1095 SbII	TMH1076	No	Carol Lewis
1097 SbII	TMH1054	No	Carol Lewis
1246 SbII	Estes	No	Sarah Sell, Nashville
659 Sc	SM72	No	Leanne Heath
702 Rc[a]	Ruggiero	Yes	Grace Leidy
1034 Sc	none	No	Porter Anderson, Cambridge
200 Rd[a]	Garfeseous	Yes	Grace Leidy
598 Rd[a]	Fidd	Yes	Grace Leidy
751 Rd[a]	Parker	Yes	Grace Leidy
703 Re[a]	Pell	Yes	Grace Leidy
1036 Se	none	No	Porter Anderson
704 Rf[a]	Dingles	Yes	Grace Leidy
706 Sf	B5693	Yes	Leanne Heath
H. parahaemolyticus 1013	T320	No	Rick Venezia, Rochester
H. parainfluenzae 700	Bossarelli	No	Grace Leidy

[a] Obtained as rough derivatives; the parental smooth cultures were all phage resistant.

incubation at 38°C, the lysed areas were removed from the plates, suspended in Eugon broth (38), and shaken with chloroform. Suspensions were then plated on the relevant strains. This procedure gave plaques for the Re strain but failed to do so (even after many efforts) for both type f cultures listed in Table 1.

Determination of modification and restriction phenotype. Glover and Piekarowicz (6) and Gromkova et al. (9) have reported on the unstable restriction and modification phenotype of the widely used strain Rd. In modification and restriction negative ($r^- m^-$) cultures, one can find many positive ($r^+ m^+$) cells and, in $r^+ m^+$ cultures, $r^- m^-$ cells may comprise as much as 10% of the population. On the other hand, $r^- m^+$ mutants are rare. My own observations agree with these reports. It thus appears that a single event can change both r and m phenotypes. I have used this phenomenon to ascertain the restriction properties of strain Rd defective lysogens by establishing their modification phenotype.

Restriction-positive strain Rd nonlysogens were isolated by streaking an r^- culture on agar and examining individual colonies for their r phenotype by determining how efficiently they plated modified and nonmodified phage HP1. Cultures were then lysogenized with *ts* phage and (after purification) their modification phenotype was confirmed by determining the plating efficiency of their *ts* phage.

Production of phage. Rapidly growing lysogens in 10 ml of Levinthal broth (38) were poured into a 9-cm petri dish and exposed to ultraviolet radiation at a dose of 600 ergs/mm^2 while the dish was swirled around. Five millimolar $MgCl_2$ (final concentration) was then added, and the suspension was further shaken at 38°C until lysis occurred. The suspensions were treated with chloroform and immediately titrated on the various strains. The phage from strain Rd and Ra cells are relatively stable, but phage from the other R strains rapidly lost its plaque-forming ability.

Efficiency of plating. Phages from the various lysogenic cultures were mixed in soft agar with about 10^8 cells of the various nonlysogens to give a multiplicity of infection of much less than 1. In the case of strain Re and RbII recipients, I used 8×10^8 bacteria and added phage at a multiplicity of infection of between 1 and 5. The plating efficiency of a given modification-type phage was obtained by comparing its plaque-forming activity on the restricting host to the activity observed on the host with the same modification and restriction phenotype (original host).

Prophage HP1 genetic map. Figure 1 gives the position of all markers used on the prophage map. It is believed that the mature phage map is very similar (35).

RESULTS

Phage HP1 restriction pattern. Table 2 shows the plating efficiency of phage HP1 from various lysogens on the various nonlysogenic strains. It can be seen that strain Rc is the only culture that allows propagation of the nonmodified phage as efficiently as does the nonrestricting strain Rd mutant. Its phage is restricted by all other strains. Strain Rc thus appears to be modification and restriction negative. This behavior was also shown by the other two strain Rc cultures studied (Table 1). Strain Rf Dingles did not support growth of phage HP1 at all. I have reported that this strain adsorbs phage readily and that about 90% of the adsorbed phage genomes are destroyed within some 30 min at 38°C (Stuy, submitted for publication). This indicates to me that this strain may have an "absolute" (i.e., extremely efficient) phage restriction system, but the presence of an interfering unrelated prophage is not yet ruled out. One other type f culture (Sf; see Table 1) behaved similarly. These observations thus disagree with an earlier report (21). The reason for this is unclear to me. The restriction behavior of the other cultures was observed with two other type a strains, two Rb, two Rd, and one Re. On the other hand, four Rb cultures appeared to possess more efficient restriction properties (Table 3). These cultures, called RbII, yielded plaques with nonmodified phage with an efficiency of about 10^{-8}. They yielded

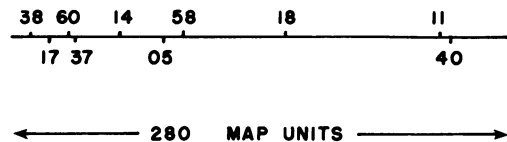

FIG. 1. *Prophage map of phage HP1.*

TABLE 2. *Phage HP1 restriction pattern in Haemophilus influenzae*

Phage from lysogenic *H. influenzae*	Modification phenotype	Efficiency of plaque formation on nonlysogens						
		Ra	RbI	Rc	Rd r^+	Re	Rf	Rd r^-
Ra Cascia	a	1.0[a]	4.6×10^{-5}	0.80	1.6×10^{-2}	4.2×10^{-9}	$<10^{-9}$	0.82
Rb Santo	bI	1.7×10^{-6}	1.0[a]	0.94	2.4×10^{-2}	$<10^{-8}$	$<10^{-8}$	0.74
Rc Ruggiero	None	9.7×10^{-7}	4.5×10^{-5}	1.0[a]	5.6×10^{-3}	$<10^{-8}$	$<10^{-8}$	0.82
Rd Garfeseous m^+	d	3.1×10^{-6}	6.9×10^{-5}	1.3	1.0[a]	1.3×10^{-8}	$<10^{-9}$	1.5
Re Pell	e	1.8×10^{-6}	3.7×10^{-5}	0.81	2.7×10^{-3}	1.0[a]	$<10^{-8}$	0.78
Rd nonrestricting m^-	None	2.2×10^{-6}	5.2×10^{-5}	1.2	8.4×10^{-2}	2.0×10^{-9}	$<10^{-9}$	1.0[a]

[a] Taken as 1.0.

TABLE 3. *Phage HP1 restriction pattern in Haemophilus influenzae Rb*

Phage from lysogenic *H. influenzae*	Modification	Efficiency of plaque formation on nonlysogens					
		RbI Santo	RbII-B5	RbII-1076	RbII-1054	RbII Estes	Rd r⁻
Rd, nonrestricting	None	3.0×10^{-5}	1.0×10^{-8}	3.5×10^{-8}	1.8×10^{-8}	0.7×10^{-8}	1.0[a]
RbI Santo	bI	1.0[a]	1.0×10^{-4}	1.0×10^{-4}	7.5×10^{-5}	3.8×10^{-5}	0.8
RbII B5-005[b]	bII	1.0×10^{-4}	1.0[a]	0.8	0.8	0.6	0.9
RbII TMH1076[b]	bII	8.3×10^{-5}	0.9	1.0[a]	1.2	ND[c]	0.8
RbII TMH1054[b]	bII	7.5×10^{-5}	1.2	1.0	1.0[a]	ND	0.8
RbII Estes[b]	bII	1.0×10^{-4}	0.9	0.9	1.1	1.0[a]	1.1

[a] Taken as 1.0.
[b] Rough non-encapsulated, phage-sensitive spontaneous mutants of the original S (smooth) strains.
[c] ND, Not determined.

TABLE 4. *Homospecific transfection (tf) and viomycin resistance transformation (vio^r) of phage HP1 nonrestricting (r^-) and restricting (r^+) H. influenzae Rd nonlysogens by DNAs from various lysogenic strains and from modified and nonmodified phage*

Donor DNA from:	Efficiency of transfection and transformation of recipient nonlysogens Rd Garfeseous			
	r⁻		r⁺	
	tf[a]	*vio*ʳ [a]	tf[a]	*vio*ʳ [a]
Rd Garfeseous (HP1⁺) m⁻	1.1×10^{-4}	0.32	$\sim 0.07 \times 10^{-4}$	0.38
Sa SM4 (HP1⁺)	0.6×10^{-4}	0.29	$< 0.02 \times 10^{-4}$	0.22
Rb Santo (HP1⁺)	2.2×10^{-4}	0.39	$< 0.02 \times 10^{-4}$	0.27
Rc Ruggiero (HP1⁺)	0.4×10^{-4}	0.45	$< 0.02 \times 10^{-4}$	0.36
Rd Garfeseous (HP1⁺) m⁺	1.0×10^{-4}	0.51	0.92×10^{-4}	0.37
Re Pell (HP1⁺)	0.4×10^{-4}	0.13	$< 0.02 \times 10^{-4}$	0.15
Phage HP1 m⁻ (from Rd)	1.0[b]		0.16	
Phage HP1 m⁺ (from Rd)	0.90		1.0[b]	
Whole phage m⁻ (from Rd)	1.0[b]		0.087	
Whole phage m⁺ (from Rd)	1.2		1.0[b]	

[a] Biological activity per donor cell or phage extracted.
[b] Taken as 1.0.

plaques with phage from the other strain RbI lysogens with a 10^{-4} efficiency, whereas RbI recipients yielded plaques with RbII-modified phage with a similar efficiency. It thus appears that there are at least two restriction phenotypes in serotype *b* cultures.

Homospecific transfection. An isogenic r⁻ and r⁺ pair of nonlysogenic strain Rd recipients was exposed to DNAs from the various lysogens, to DNA extracted from modified and nonmodified phage, and to whole modified and nonmodified phage (to check the r phenotype of the recipient cultures). The results are expressed as transfecting activity per donor cell or phage extracted. It can be seen in Table 4 that the r⁺ recipient did restrict all transfecting DNAs extracted from phage or from lysogenic cultures with no (Rd m⁻) or with a different phage HP1 modification system. These results thus confirm the report by Gromkova et al. (9) that transfecting DNA from r⁻ m⁻ lysogens is restricted by Rd r⁺ nonlysogens. Transformation, on the other hand, was not affected by the r⁺ phenotype of the recipient cells (see also below). Similar conclusions have been published by Gromkova and Goodgal (7, 8) for *Haemophilus* and by Trautner et al. (39) for *B. subtilis*.

To establish whether adsorbed nonmodified phage DNA is degraded more extensively in r⁺ recipients, ^{32}P-labeled nonmodified phage HP1 DNA was adsorbed by the above two recipients, and the acid-soluble breakdown was measured (34). It can be seen in Fig. 2 that the two recipients behaved similarly. Thus, the initial solubilization of adsorbed DNA, as described in an earlier publication (34), is not influenced by the absence of certain DNA modifications.

Homospecific transformation. Isogenic r⁻ and r⁺ pairs of temperature sensitive (ts) defective phage HP1 Rd lysogens were exposed to DNAs extracted from lysogenic cultures of the various serotype strains each possessing an independent spontaneously acquired viomycin resistance (vio^r) mutation. The modification and

restriction phenotype of the recipients was established by plating their spontaneously released ts phage on the r⁻ and r⁺ nonlysogens mentioned in Table 4. Presumably, the lysogenic derivatives had thus not lost their restriction phenotype (6, 9; see also Materials and Methods). Table 5 gives the efficiencies of transformation, expressed as activity per extracted donor cell, for the *vio*ʳ markers and for the two prophage markers studied. It can be seen that the recipient r phenotype appeared to have little if any influence on the efficiency of viomycin resistance of prophage transformation. The observed small differences in *vio*ʳ transformation between the various strains may reflect a certain degree of nonhomology between donor and recipient DNA (10). Genetic transformation in *B. subtilis* (39) and in *Haemophilus* (7) has also been reported to be independent of recipient r phenotype.

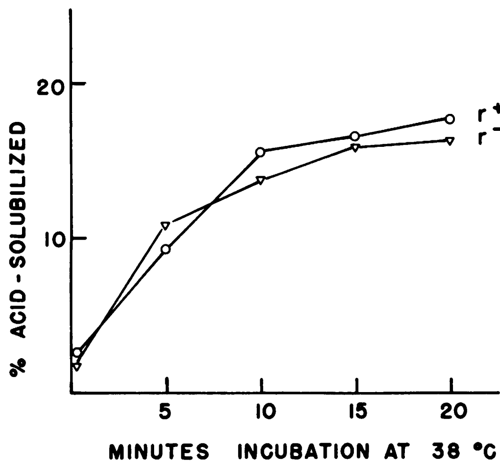

FIG. 2. *Production of acid-soluble breakdown products from adsorbed nonmodified ³²P-labeled phage HP1 DNA by restricting (r⁺) and nonrestricting (r⁻) H. influenzae Rd recipients. Competent cells were mixed with the DNA for 5 min at 30°C, chilled, and collected by centrifugation. They were resuspended in fresh broth and shaken at 38°C. DNA uptake was 35%; multiplicity of infection was 0.6. Samples were taken at various times and analyzed as described (34) for acid-soluble radioactivity.*

Heterospecific transformation. *H. parainfluenzae* Bossarelli (as well as four others examined) is resistant to phage HP1. All efforts to prepare an HP1 lysogen through transfection failed. The *H. parahaemolyticus* strain listed in Table 1 was found to be defectively lysogenic for phage HP1 (Stuy, submitted for publication). It was transformed to streptomycin resistance using the *H. influenzae str*ʳ marker (38) and then to a wild-type HP1 lysogen using purified nonmodified phage HP1 DNA. Phage from a spontaneous *vio*ʳ mutant behaved as nonmodified when plated on the various recipients listed in Table 2. In Table 6, I have summarized the transformation results using *H. parahaemolyticus* lysogen donor DNA and two isogenic r⁻ and r⁺ pairs of defective HP1 Rd lysogens as recipients. The transformation efficiencies of the *str*ʳ and *vio*ʳ markers were very low, as is expected for heterospecific transformation. The transformation efficiencies for the prophage markers were only some 90% lower for both r⁻ and r⁺ recipients. To examine whether possible nonhomology in the bacterial DNA flanking the HP1 prophage was responsible for that reduction, I exposed a number of different r⁻ defective HP1 Rd lysogens to the *H. parahaemolyticus* DNA and scored *vio*ʳ and wild-type prophage transformants. The results presented in Table 7 indicate that the prophage marker location was of little importance for its transformation efficiency. Thus, the terminal NG138⁺

TABLE 5. *Prophage and viomycin resistance (vio*ʳ*) transformation of phage HP1 modification and restriction negative (m⁻ r⁻) and positive (m⁺ r⁺) H. influenzae Rd Garfeseous ts HP1 defective lysogens by DNAs from various lysogens*

Donor DNA from:	Efficiency of HP1 prophage and *vio*ʳ transformation of recipient defective Rd HP1 lysogens							
	NG158				NG160			
	r⁻ m⁻		r⁺ m⁺		r⁻ m⁻		r⁺ m⁺	
	Prophage[a]	*vio*ʳ [a]	Prophage[a]	*vio*ʳ [a]	Prophage[a]	*vio*ʳ [a]	Prophage[a]	*vio*ʳ [a]
Rd Garfeseous (HP1⁺) m⁻	0.10	0.33	0.11	0.33	0.12	0.40	0.098	0.34
Sa SM4 (HP1⁺)	0.18	0.16	0.26	0.17	0.12	0.14	0.092	0.13
Rb Santo (HP1⁺)	0.28	0.28	0.34	0.24	0.12	0.30	0.11	0.20
Rc Ruggiero (HP1⁺)	0.16	0.29	0.21	0.33	0.26	0.30	0.21	0.20
Rd Garfeseous (HP1⁺) m⁺	0.30	0.37	0.33	0.38	0.24	0.38	0.19	0.52
Re Pell (HP1⁺)	0.10	0.096	0.16	0.068	0.054	0.042	0.048	0.040

[a] Biological activity per donor cell extracted.

TABLE 6. *Homospecific and heterospecific prophage, viomycin resistance (vio^r), and streptomycin resistance (str^r) transformation of phage HP1 nonrestricting (r^-) and restricting (r^+) H. influenzae Rd ts HP1 defective lysogens by DNA extracted from lysogenic H. influenzae Rd and from lysogenic H. parahaemolyticus*

Donor DNA from:	RD recipient defective HP1 lysogen	Efficiency[a] of transformation					
		r^-			r^+		
		Prophage	str^r	vio^r	Prophage	str^r	vio^r
Rd Garfeseous ($HP1^+$) m^-	NG158	0.13	0.30		0.11	0.28	
H. parahaemolyticus ($HP1^+$) m^-		0.019	1×10^{-3}	6×10^{-4}	0.015	8×10^{-4}	4×10^{-4}
Rd Garfeseous ($HP1^+$) m^-	NG137	0.071	0.25		0.060	0.19	
H. parahaemolyticus ($HP1^+$) m^-		0.0082	6×10^{-4}	2×10^{-4}	0.0066	1×10^{-3}	5×10^{-4}
Rd Garfeseous ($HP1^+$) m^-	NA118	0.060	0.30				
Vortex mixed		0.065	0.24				
Needle sheared		0.043	0.075				
H. parahaemolyticus ($HP1^+$) m^-		0.019	3×10^{-4}				
Vortex mixed		0.021	5×10^{-4}				
Needle-sheared		0.025	3×10^{-4}				

[a] Biological activity per donor cell extracted.

TABLE 7. *Heterospecific prophage and viomycin resistance (vio^r) transformation of nonrestricting phage HP1 defective Rd lysogens with DNA from lysogenic H. parahaemolyticus*

Marker	Efficiency[a] of transformation of defective lysogens								
	NG138[b]	NA117[b]	NG137[b]	NA114[b]	NG105[b]	NA118[b]	NG111[b]	Triple[c]	*H. parainfluenzae*[d]
Prophage	0.011	0.016	0.0085	0.016	0.021	0.010	0.0083	0.00085	
vio^r	5×10^{-4}	8×10^{-4}	8×10^{-4}	4×10^{-4}	3×10^{-4}	3×10^{-4}	6×10^{-4}	7×10^{-4}	0.13

[a] Biological activity per donor cell extracted.
[b] Singly defective lysogen each having a different prophage marker (see map in Fig. 1).
[c] Triply defective lysogen NG137NA118NG140 (the cotransformation efficiency relative to the activity of the NA118 marker is 0.25 in homospecific crosses).
[d] Nonlysogen.

marker transformed as efficiently as the central marker $NA118^+$, which presumably is situated in the middle of a long sequence of homologous DNA. It is possible, of course, that there is no, or little, nonhomology between donor and recipient bacterial DNA at the left end of the HP1 prophage. Shearing the *H. parahaemolyticus* DNA to *Escherichia coli* phage λ-sized fragments (they co-sedimented with phage λ DNA in sucrose density gradients) by treating it intensively with a Vortex mixer at a maximum setting for 60 s did not significantly increase its prophage transformation activity. Shearing the DNA by forcing it through a 24-gauge needle seven times also did not change its activity (Table 6).

Heterospecific transfection. Heterospecific transfection was carried out by exposing nonlysogenic *H. influenzae* strain Rd and *H. parainfluenzae* competent cells to DNA extracted from lysogenic *H. parahaemolyticus* and by exposing competent nonlysogenic *H. parainfluenzae* to DNA from lysogenic *H. influenzae* Rd. *H. parainfluenzae* recipients were diluted fivefold before mixing them in soft agar with Rd phage HP1 indicator cells. It can be seen in Table 8 that all heterospecific transfection crosses were very inefficient; they were reduced below the minimum measurable level of about 5×10^{-7} transfection activity units per extracted donor cell (see also reference 7). Nonmodified phage HP1 DNA from Rd lysogens transfected *H. influenzae* and *H. parainfluenzae* with nearly equal efficiency. This indicates that *H. parainfluenzae* does not restrict phage HP1 and thus has the r^- m^- phenotype (see also reference 9).

DISCUSSION

The data listed in Tables 2 and 3 show that all *H. influenzae* serotype strains have a different phage HP1 restriction phenotype. It should be noted that these differences in behavior are probably caused by their different class I restriction enzymes (requiring Mg ions, *S*-adenosylmethionine, and adenine triphosphate) (see

TABLE 8. *Homospecific and heterospecific prophage transfection (tf) and transformation (str^r and vio^r) of nonrestricting H. influenzae Rd and of H. parainfluenzae recipients with DNA extracted from lysogenic H. influenzae Rd, from lysogenic H. parahaemolyticus, and from nonmodified mature phage*

Donor DNA from:	Efficiency[a] of transfection and transformation of nonlysogenic recipient			
	Rd Garfeseous		*H. parainfluenzae*	
	tf	str^r	tf	vio^r
Rd Garfeseous (HP1$^+$) m$^-$	5.0×10^{-5}	0.16	$<5 \times 10^{-7}$	
H. parahaemolyticus (HP1$^+$)	$<5 \times 10^{-3}$	1×10^{-3}	$<5 \times 10^{-7}$	0.080
Mature phage HP1 m$^-$	2.1×10^{-5}		1.4×10^{-5}	

[a] Biological activity per donor cell or phage extracted.

below). Each restriction enzyme is possibly paired with a similarly site-specific DNA modification enzyme (methylase?). If that is the case, Ra and Re strains, and perhaps RbII ones as well, may each have two different modification enzymes (20, 21), whereas Rd and RbI strains may each have one modification enzyme (6, 9, 22). Rc strains have no such enzyme, whereas Rf strains may have more than one. Various DNA methylase activities have been detected in *H. influenzae* (24–26), and it would not be surprising if the modification enzymes were indeed DNA methylases. In addition to class I enzymes, the various *H. influenzae* serotype strains, as well as *H. parainfluenzae* and *H. parahaemolyticus*, have certain class II site-specific endonucleases (requiring only Mg ions). Ra and Re strains do not have such enzymes (R. J. Roberts, personal communication), and Rd has two different class II endonucleases called *Hind* II and *Hind* III (12, 13, 19, 32), whereas Rc strains have only the *Hinc* II enzyme (isoschizomer of *Hind* II) (13), and RbI strains have only the *Hinb* III enzyme (isoschizomer of *Hind* III) (R. J. Roberts, personal communication). Rf strains have *Hinf* endonuclease I, whereas *H. parahaemolyticus* has Hph I (R. J. Roberts, personal communication). Finally, *H. parainfluenzae* has two different class II restriction enzymes, Hpa I and Hpa II (5, 24, 30). If each class II endonuclease is paired with a similarly site-specific DNA modification enzyme (methylase?), it is clear that the various strains used in this study probably have very different DNA modification sequences. The observation that Rc strains do not restrict phage HP1 grown on Ra and Re hosts and the observation that *H. parainfluenzae* is efficiently transfected by nonmodified Rd-grown phage HP1 DNA indicate strongly that class II endonucleases are not involved in phage HP1 restriction.

Despite all these possible different modification sequences between donor and recipient DNA, the results of the crosses outlined in this study give no indication that restriction enzymes of either class I or class II play a significant role in the determination of the efficiency of homospecific and heterospecific transformation. This is in agreement with published statements by Gromkova and Goodgal (7) that *Haemophilus* transforming DNA is not excluded by class I or II endonucleases in crosses between closely related species. Since my experiments showed that the transforming DNAs used were sensitive to restriction by r^+ recipients (in transfection), I conclude that DNA modification (methylation?) patterns are of little significance in genetic transformation.

My observations and those of others (7, 30) about reduced HP1 phage and HP1 prophage DNA transfection in homospecific r^+ Rd recipients indicate that restriction of transfecting DNA is probably the same phenomenon as phage restriction and is only caused by class I restriction enzymes. Since I also observed that nonmodified phage DNA was made acid soluble equally rapidly in both r^- and r^+ nonlysogenic Rd recipients, I conclude that transfection restriction may act only on those few phage DNA molecules that enter the replication process. Further observations that foreign DNAs such as those from *E. coli* phage λ (14) and T7 (unpublished data), or from *E. coli* cells (34), persist for one or more generation times after adsorption by *H. influenzae* Rd cells support this view. It is then unlikely that restriction enzymes function as salvaging factors in the elimination of penetrated foreign DNA.

The heterospecific transformation crosses showed that DNA homology between donor and recipient DNA is an overriding factor in the eventual DNA integration process. Homologous prophage markers showed transfer efficiencies several orders of magnitude higher than those observed for antibiotic resistance markers in the same crosses, and the efficiencies of prophage marker transfer were not much lower than those scored for homospecific prophage transformation.

I believe that my experiments show that DNA homology may also be important in transfection. The homospecific transfection experiments showed that the source of donor prophage DNA is not a significant factor for the transfection efficiency when r^- recipients are used. In heterospecific transfection, however, this efficiency was very much reduced. One might visualize in homospecific transfection that the bacterial donor DNA regions flanking the prophage can "line up" with the corresponding DNA regions in the recipient. This event may stimulate subsequent steps leading to the excision of the phage genome. No such alignment may occur in heterospecific transfection. I have tried to generalize this conclusion by studying heterospecific transfection of *H. influenzae* Rd recipients with *H. parainfluenzae* or *H. aegyptius* wild-type prophage DNA. Unfortunately, the defective lysogens of phage HP1 in my culture collection could not be converted to wild-type lysogens.

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ADDENDUM IN PROOF

Very recently an Sb strain was obtained that, after treatment with nitrosoguanidine, yielded a number of rough segregants which did not restrict or modify phage HP1. There may thus be three restriction phenotypes in type b strains.

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