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The contribution of plasmid and host genes to plasmid-mediated interference with phage growth

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SUMMARY

The R factors, R56 and R64, interfere with the growth of several otherwise unrelated phages, including λ, P22 and T7. In addition, *λvir* is inhibited more strongly in λ lysogens carrying either R factor when the prophage has an intact O – P region. Interference does not occur in certain *strA* R+ hosts but may reappear if they also carry other mutations affecting ribosomal proteins. Host mutants not permitting interference were isolated but were not *strA*. Non-interfering plasmid mutants were also isolated, of which some also failed to cause interference after transfer to a different host genus.

1. INTRODUCTION

Many types of plasmid interfere with phage infection by processes other than restriction or repression. The two R factors discussed here, R56 and R64, belong to Group V of Bannister & Glover (1968) and are of particular interest because they interfere with two coliphages, φI and λ, which are not generally thought of as related, as well as with phages P22 and St in *Salmonella typhimurium* LT2. Interference depends on functions provided by both plasmid and host which can be distinguished genetically. Non-interfering plasmid mutants have been isolated; and wild type plasmids do not cause interference in certain streptomycin-resistant hosts. In addition, interference with phage *λvir* is increased if the host also carries a λ prophage with an intact O – P region.

2. MATERIALS AND METHODS

(i) *Culture Media.* Oxoid Blood Agar Base (CM55). Oxoid Nutrient Broth No. 2 (CM67). YE broth (Hardy, Harwood & Meynell, 1974).

(ii) *Phages.* For φI, φII, T3, T7, H and W31, see Williams & Meynell (1971). Stocks were prepared in 1 M-NaCl to prevent spontaneous inactivation (Hausmann, personal communication). Lysogens carrying deleted λ prophages were generously provided by Dr Allan Campbell; *λvir* was obtained from Dr Richard Hayward; phage P22c_2 was kindly provided by Dr Myron Levine. Phage St was isolated from the River Stour, Kent, by incubating river water with strain M525, a P22 lysogen of *Salmonella typhimurium* LT2. This phage differs from P22 in its

virulence, long tail, immunity pattern and antigenic specificity. Titrations were performed as in Williams & Meynell (1971).

(iii) *Bacteria.* For *Escherichia coli* K-12, see Bachmann (1972). For *E. coli* K-12 HfrC, strain D7001, and for strains of *E. coli* B, kindly provided by the late Dr Luigi Gorini, see Breckenridge & Gorini (1970) and Chakrabarti & Gorini (1975*a*, *b*). For *Salmonella typhimurium* LT2, strain SR120, cured of a B prophage, see Zinder (1958); and for strain M827, which is LT2 cured of a cryptic plasmid, see Spratt, Rowbury & Meynell (1973). The source of the R factors was *E. coli* K-12, J5-3, carrying R56 or R64 (i.e. M2219 and M2221, respectively). Both R factors are self-transmissible, *fi*$^-$ and specify tetracycline resistance (Lawn *et al.* 1967; Hedges, 1972). R64 also specifies low-level streptomycin resistance and its hosts appear sensitive when tested against discs containing 2000 μg streptomycin.

(iv) *Mutagenesis.* Ethyl methane sulphonate (EMS) was used, as in Meynell & Meynell (1970).

(v) *Isolation and scoring of* strA *mutants.* Spontaneous mutants were isolated by centrifuging 100 ml shaken overnight cultures in YE broth + 0·2 % (w/v) glucose, resuspending the cells in 1 ml of YE broth and spreading 0·5 ml on nutrient agar containing 200 μg/ml streptomycin. Mutants from EMS-treated cultures were isolated on the same agar but without concentrating the cells. Streptomycin-resistant and dependent mutants were distinguished by streaking 1/100 dilutions of overnight broth cultures on ditch plates with 200 μg/ml streptomycin in the ditch.

Non-interfering mutants. When approximately 400 wild type R$^-$ cells are spread on plates previously spread with *ca.* 10^4 virulent phage (e.g. T7, P22c_2 or St), virtually all form colonies but each colony is nibbled. With less phage, colonies appear normal; with more phage, fewer colonies form and most are heavily eroded. R$^+$ colonies appear normal. Non-interfering mutants of R$^+$ strains can therefore be identified by their nibbled colonies formed in the presence of *ca.* 10^4 phage/plate (Morrison & Malamy, 1971).

3. RESULTS

The observations are largely concerned with efficiencies of plating (E.O.P.) of phages λ, P22 and T7 on strains carrying either R56 or R64. However, as R56 lowered the E.O.P. more markedly than R64, the majority of results were obtained with R56. The occurrence of interference is detectable not only by a lowered E.O.P. but even more readily by an altered plaque morphology. A normal plaque of T7 has a clear centre with a pronounced halo but, as the E.O.P. begins to decrease, the halo shrinks and then disappears, and the centre of the plaque becomes smaller and turbid.

(i) *Phages λ, P22 and T7*

Phage λ. Previous tests with λ all appear to have used λvir plated on λ lysogens such as *Escherichia coli* K-12, strains J5-3 (Bannister & Glover, 1968) or J6-2 (Hedges, 1972). However, on plating λvir on λ^- hosts, both R$^-$ and R$^+$, it became apparent that the E.O.P. on the R$^+$ hosts was lowered by the λ prophage. Thus, the

E.O.P. of λ*vir* on *E. coli* K-12 strain C600 and its R56+ derivatives were: λ−R− (1·0); λ+R−, 1·0; λ−R+, 0·23; and λ+R+, 0·08.

The region of the λ prophage producing this effect was determined by plating λ*vir* on R56+ derivatives of various λ lysogens carrying partial deletions of the prophage. The results indicated that an intact O – P region in the prophage was required (Fig. 1).

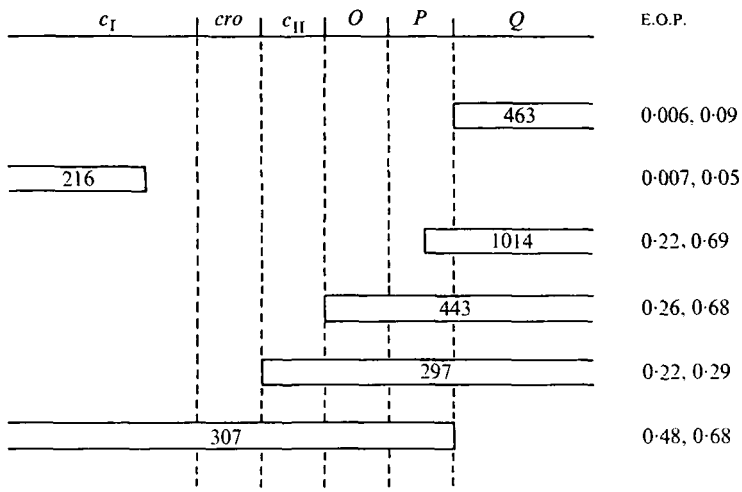

Fig. 1. Joint effect of the λ prophage and of R56 in lowering the E.O.P. of phage λ*vir*. The Figure shows part of the λ map and the regions deleted in the six defective lysogens examined. The strain numbers are those of Dr Allan Campbell. The pairs of E.O.P. values came from duplicate independent titrations.

Phage P22. The interfering action of R56 was not confined to *Escherichia coli*. The E.O.P. of either P22c_2 or of the unrelated phage St on R56+ or R64+ derivatives of *Salmonella typhimurium* LT2 was $< 10^{-6}$.

Phage T7. Bannister & Glover (1968) found that phage φI was inhibited by R56. Since φI and T7 are very closely related (Hyman, Brunovskis & Summers, 1974), the expectation was that T7 would also be inhibited. In fact, its E.O.P. on the R+ indicators was reduced to 10^{-5}, and T7 was subsequently used rather than φI.

(ii) *Streptomycin-resistant and dependent hosts*

While plating T7 on a number of R+ derivatives of *Escherichia coli* K-12, its E.O.P. was found to be far higher on certain streptomycin-resistant (*strA*) mutants than on their streptomycin-sensitive parents; e.g. 10^{-1} compared to 10^{-5}. This was confirmed using *strA* mutants from two different lines of K-12 (namely, strain AB1157 descended from Y53, and W2637 descended from W1485: Bachmann, 1972), since the Y53 line is anomalous in its relation to female-specific phages (Williams & Meynell, 1971). The same increased E.O.P. was also found with other phages related to T7, including φI, φII, W31, T3 and φH. Hence, R56, although *fi*− and specifying I-like sex pili (Lawn *et al.* 1967), resembled F which also permits efficient plating of T7 in certain *strA* hosts (Chakrabarti & Gorini, 1975*a*).

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Genetic and physiological analysis of *strA* mutants of *Escherichia coli* B divides them into four classes, represented by *strA1*, *strA2*, *strA40* and *strA60* (Breckenridge & Gorini, 1970). The plating of T7 on R56+ derivatives of two of these classes was as follows. The E.O.P. on all the R− strains equalled that on R− K-12; the E.O.P. on the R+ *strA*+ and *strA40* strains was 10^{-6}; but the E.O.P. on the R+ *strA1* strain was increased to 10^{-2}. Plating on a fourth strain, *strA1 ram*-1 *spc*-1 ($< 10^{-6}$), will be discussed later.

Table 1. *Plating of phage T7 on streptomycin-resistant and dependent mutants of* Escherichia coli *K-12 carrying wild type R56*

Streptomycin phenotype	Mutagen	Culture no.	Colonies tested per culture	Colonies with E.O.P.					
				10°	$\geqslant 10^{-1}$	$\geqslant 10^{-2}$	$\geqslant 10^{-3}$	$\geqslant 10^{-4}$	$\geqslant 10^{-5}$
Resistant	None	1	8	—	2	2	2	1	1
		3	3	—	—	—	—	2	1
		4	9	—	—	—	—	8	1
		5	4	—	—	—	1	2	1
	EMS	1–11	1	—	—	1	—	7	3
Dependent	None	7	2	—	2	—	—	—	—
	EMS	7–12	1	—	1	4	—	—	1

EMS: ethyl methane sulphonate.
E.O.P. on *strA*+ R+ culture: 10^{-5}.

A series of *strA* mutants of K-12 were then isolated, including streptomycin-dependent as well as resistant strains, both spontaneous and induced with ethyl methane sulphonate (EMS). The latter was included because it alters the incidence of the four types of *strA* mutant in *Escherichia coli* B (e.g. *strA1* comprises 6 % of EMS-induced mutants compared to 58 % found spontaneously, as opposed to *strA60* which comprises 54 % of EMS mutants compared to only 4 % spontaneously: Table 4, Breckenridge & Gorini, 1970). Table 1 summarizes the results of plating T7 on their R56+ derivatives. It should be noted that these values using mutants of *E. coli* K-12 isolated on 200 μg/ml streptomycin may not be directly comparable to those of Gorini and his colleagues using mutants of *E. coli* B isolated on 500 μg/ml. Table 1 shows that the E.O.P. did not fall into discrete classes but were between 10^{-5}, the value for a *strA*+ host, and a maximum of 0·2. Spontaneous resistant mutants from a given culture frequently differed (e.g. cultures 1 and 5). Inhibition by R56 was generally far less marked in dependent than in resistant mutants.

(iii) *Mutants not inhibiting phage growth*

If about 400 cells of an R+ culture are spread on nutrient agar with 10^4–10^5 virulent phage, most will form colonies of which the great majority appear normal. However, a small minority of colonies are nibbled and, on subculture, some are found to be mutants no longer inhibiting phage growth (Morrison & Malamy, 1971).

In this way, examination of *ca.* 10^5 colonies yielded 28 mutants from *Escherichia coli* K-12 exposed to T7, and 12 from *Salmonella typhimurium* LT2 exposed to P22c_2 or St.

Such mutants might be either plasmid or chromosomal. Plasmid mutants can be detected by testing their ability to interfere after transfer to a new host. Of 13 mutants thus examined, Table 2 shows that 7 appeared to be plasmid (of which at least 4 were independent), Moreover, such transfers showed that a mutant plasmid like R64-7 selected in *Escherichia coli* K-12 by phage T7 could also behave as mutant in *Salmonella typhimurium* LT2 when tested with phage P22. Conversely, a mutant selected in LT2 could also appear mutant in K-12.

Table 2. *Independent plasmid mutants*

		E.O.P.*		
			After transfer to	
Plasmid no.	Selected by phage	In original host	Same species	Alternative species
R56–7†	T7	0·2	1·0	$<10^{-6}$
R64–1†	T7	0·1	0·1	$<10^{-5}$
R64–7	T7	0·5	0·03	1·0
R64–8†	P22c_2	1·0	1·0	10^{-2}

* Titrations: T7 in *E. coli*; P22c_2 and St in *Salm. typhimurium*. E.O.P. with wild type plasmids: $= 10^{-5}$ to 10^{-6}. P22 and St gave the same E.O.P.

† Two mutant colonies tested from each mutagenized culture, with identical results.

Chromosomal mutants can be detected, in principle, by curing strains of their R factors (which is not always feasible) and by reintroducing the wild type R factor. An alternative approach of greater interest, in view of the above findings with *strA* mutants, is that chromosomal mutants might simply carry mutant *strA* alleles. This should be readily detectable by the response of the strains to streptomycin: resistant mutants are totally resistant to sensitivity disks impregnated with 2000 μg streptomycin, whereas strains carrying the wild type R factors show large inhibition zones having a radius of 15 mm from the edge of the disk. On testing the 28 mutant clones, no diminution in streptomycin sensitivity was found compared to their parent.

Wild type F still caused interference in the presence of a non-interfering R mutant. Thus, phage T7 had an equally low E.O.P. ($< 10^{-5}$) on HfrC (strain D7001 of Chakrabarti & Gorini, 1975*a*) whether it was R$^-$ or carried a non-interfering mutant of R56 or R64.

4. DISCUSSION

Interference with phage growth caused by R56 or R64 presents clear similarities to that produced by F. Two striking features of the present results are the lack of specificity of interference (Table 2) and the influence of the host *strA* locus, both of which presumably reflect the underlying mechanism. The role of the *strA* locus

is not at present understood but is presumably mediated through the 30S ribosomal subunit. This interpretation is supported by the finding that the mutations, *ram-1 spc-1*, which alter proteins of this subunit other than those determined by *strA*, abolished the increase in E.O.P. produced by *strA1*. Rifampicin-resistant mutations may have the same effect, indicating that the increased plating due to *strA1* can also be counteracted by an appropriate alteration in RNA polymerase (Chakrabarti & Gorini, 1975*b*). Moreover, two chromosomal mutations mapping near *strA* permit phage T5 to grow on hosts carrying colicin factor Ib-P9 (Hull & Moody, 1976).

Two classes of hypothesis have been suggested to explain these instances of interference: either a specific mechanism like blocked transcription (Moyer, Fu & Szabo, 1972) or blocked translation of late phage mRNA (Morrison & Malamy, 1971; Blumberg, Mabie & Malamy, 1976); or a generalized breakdown in cell function (Ponta *et al.* 1975; Britton & Haselkorn, 1975; Yamada & Nakada, 1975; Condit, 1976). On either hypothesis, interference would be expected to occur in a host carrying wild type F and mutant R, as indeed it does. Our finding that a non-interfering mutant of R64 selected in *Escherichia coli* by T7 also failed to interfere with P22 in *Salmonella typhimurium* suggests a non-specific mechanism. One possibility is that plasmid products (pilus protein has been suggested by Ponta *et al.* 1975) alter the host cell membrane which breaks down prematurely after phage infection. However, the fact that non-interfering mutants are still self-transmissible suggests that the hypothetical product is not involved in conjugation.

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