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Bacteriophage T4 resistance to lysis-inhibition collapse

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Summary

Lysis inhibition is a mechanism of latent-period extension and burst-size increase that is induced by the T4 bacteriophage adsorption of T4-infected cells. Mutants of T4 genes *imm*, *sp* and *5* (specifically the *ts1* mutant of *5*) display some lysis inhibition. However, these mutants experience lysis-inhibition collapse, the lysis of lysis-inhibited cells, earlier than wild-type-infected cells (i.e. their collapse occurs *prematurely*). Lysis from without is a lysis induced by excessive T4 adsorption. Gp*5* is an inducer of lysis from without while gp*imm* and gp*sp* effect resistance to lysis from without. This paper shows that interfering with the adsorption of phages to *imm*-, *sp*- or *5ts1*-mutant-infected cells, in a variety of contexts, inhibits premature lysis-inhibition collapse. From these data it is inferred that wild-type T4-infected cells display resistance to lysis-inhibition collapse by a mechanism resembling resistance to lysis from without.

1. Introduction

Bacteriophage (phage) therapy continues to represent a promising but only minimally explored alternative to conventional chemotherapeutic treatments of bacterial disease (Levin & Bull, 1996; Lederberg, 1996; Barrow & Soothill, 1997; Alisky *et al.*, 1998; Kutter, 1998). In all treatments of bacterial disease, it is wise to choose one's antimicrobials on the basis of a robust understanding of properties and potential efficacies. Phages are far more complex than chemical antimicrobials, making their characterization much more difficult. It has been suggested that rational design of phage therapies should include some consideration of phage virulence (capacity to kill bacteria). Efforts aimed at characterizing phages for use as antibacterial agents might therefore concentrate on those phages displaying the greatest virulence (Kutter, 1998). The T-even phages of *Escherichia coli* B (Demerec & Fano, 1945) display exceptional virulence, and are of a type that appears to be very common in nature (Ackermann & Dubow, 1987). Here, we explore aspects of the physiology and genetics of lysis inhibition (LIN), a distinctive, virulence-affecting quality of the lytic cycle of T-even phages, as displayed by phage T4.

LIN is a mechanism of burst-size increase and latent-period extension induced by T4 secondary adsorption of T4-infected cells (Doermann, 1948; Rutberg & Rutberg, 1965; Bode, 1967; Abedon, 1994; Asami *et al.*, 1997; Paddison *et al.*, 1998). This inducible growth strategy is an adaptation to environments containing high densities of T4-infected cells (Abedon, 1990), and occurs in a manner somewhat analogous to the autoinduction phenomenon seen in bacteria (Kaiser, 1996). When T4-infected-cell density is high, high densities of free phages are generated, uninfected cells are rapidly infected, secondary adsorption is likely, and LIN is induced with high probability (Abedon, 1990, 1992, 1994).

LIN lies at an interface between whole-organismal biology and molecular genetics. On the one hand, we may at least envisage an eventual molecular understanding of the phenomenon (perhaps analogous to our high level of understanding of many aspects of T4 biology; Mathews, 1994). On the other hand, LIN represents, minimally, an interaction between three whole organisms: a bacterium, a phage that is infecting the bacterium, and a second phage that is attempting to infect the bacterium. Indeed, the LIN phenotype is manifest, in both liquid and solid medium, as a highly social, population-wide phenomenon (Abedon, 1990, 1992). As a consequence, in defining LIN, a purely molecular characterization is insufficient and certainly is limited in its ability to shed light on the ecological

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significance of the phenomenon, e.g. with regard to its potential impact on antibacterial therapy.

This study undertakes a whole-organismal approach towards characterizing the phenotypes associated with three T4 genes whose mutants are known (or shown here) to be defective in LIN: *imm*, *sp* and *5*. Phage mutations affecting LIN were classified by Hershey (1946*b*) into three phenotypic categories. First are the *r* mutants, which prevent LIN altogether (mutations in so-called rapid-lysis loci such as *rI* or *rII*; e.g. Paddison *et al*., 1998). Secondly, and less well known or well characterized, are the *w* mutations, or weak inhibitors, which are only partially defective in LIN. Mutations in T4 genes *sp*, *5* and *imm* (the latter as shown here) display the *w*-mutant phenotype, allowing some LIN, but not as much LIN as that displayed by wild-type T4 phages (Emrich, 1968; Cornett, 1974; Kao & McClain, 1980*b*). Thirdly, and similarly obscure, are the *s* mutations, or *strong* lysis inhibitors, which display more LIN than wild-type. In an unfortunate coincidence, *s* was also used by Emrich to abbreviate *spackle*, the gene she discovered, which here is abbreviated *sp*. That is, as noted, the *spackle* mutant phenotype, in the Hershey scheme, is *w*-like rather than *s*-like.

Genes *imm*, *sp* and *5*, in addition to contributing to wild-type LIN, also have an effect on T4 lysis from without (LO) and T4 adsorption (as reviewed in Abedon, 1994). Gene *5* serves as an effector of these latter phenotypes. The wild-type 5 protein is a lysozyme that is a component of the tail tube tip of virion particles (Kao & McClain, 1980*a*; Nakagawa *et al*., 1985; Mosig *et al*., 1989; Takeda *et al*., 1998). During adsorption, the phage tail tube is responsible for the transfer of DNA from the extracellular phage head to the adsorbed-cell cytoplasm (Coombs & Arisaka, 1994; Goldberg *et al*., 1994). Knock-out of gene *5* results in aberrant adsorption (Nakagawa *et al*., 1985). Following high-multiplicity phage adsorption, virion-associated gp*5* induces LO, a virion particle/extracellularly-induced lysis that can occur even in the absence of phage infection (Kao & McClain, 1980*b*; Nakagawa *et al*., 1985; Abedon, 1994; Tarahovsky *et al*., 1994; Asami *et al*., 1997). Genes *imm* and *sp*, by contrast, serve as both LO and adsorption inhibitors. Mutation in either gene results in a defect in resistance to LO. Gene *imm* and *sp* mutants are additionally defective in superinfection exclusion. In wild-type phages, superinfection exclusion is manifest as an inhibition of proper secondary phage adsorption (reviewed in Abedon, 1994).

It is not certain how *imm* expresses its associated phenotypes, though Imm protein may interfere with the otherwise normal interaction between adsorbing phages and the *E. coli* inner membrane (Lu & Henning, 1989; Lu *et al*., 1993). The *sp* gene product is thought to act by interfering with gp*5*-mediated murein hydrolysis. This mechanism was proposed by Kao & McClain (1980*a*, *b*), who isolated a gene-*5* temperature-sensitive mutant, *5ts*1, that mimics many *sp*-mutant phenotypes. They hypothesized that the *5ts*1 mutation imparts to free phages an ability to bypass gp*sp*. For example, *5ts*1-mutant phages show an enhanced propensity to induce LO upon adsorption to wild-type-infected cells. The *5ts*1 mutation thus codes for a mutated gain in function (e.g. enhanced induction of LO), albeit at a cost of high-temperature growth.

Gene products Sp and 5 additionally play complicated, and to some extent artifactual, roles in lysis from within (LI) – the T4 lysis that occurs at the end of a normal T4 latent period (not lysis-inhibited) which is, in part, induced by the T4 E protein, the primary T4 lysozyme (Mosig *et al*., 1989). Lethal mutations of gene *e*, but not those of gene *5*, confer a defective LI. The double mutant *e sp*, however, displays a sufficiently effective LI to allow plaque formation (Emrich, 1968). Suppression of *e* mutations by *sp* mutations probably occurs because cytoplasmic gp*5* can digest the cell wall from within, but only if gp*sp* is absent. Consistent with this hypothesis, Kao & McClain (1980*a*) showed that the *5ts*1 mutation can suppress *e* mutations. Extrapolating from this observation, they additionally suggested that the intracellular lytic action of gp*5* might explain why cells infected with *sp* or *5ts*1 mutants display a defective LIN (Kao & McClain, 1980*b*).

Induction of LIN requires secondary adsorption and various wild-type alleles (reviewed by Abedon, 1990, 1994; Paddison *et al*., 1998). Secondary adsorptions also induce and propagate the reversal of LIN, a relatively speedy, population-wide lysis called LIN collapse (Abedon, 1992). As far as this author knows (Kao & McClain's speculation aside, 1980*b*), no other T4, *E. coli* or environmental component has been demonstrated to play a causative role in LIN collapse (Young, 1992; Abedon, 1994), at least during constant-temperature growth (i.e. shifts to low temperatures can also induce LIN collapse, which perhaps acts as a novel mechanism of T4 lysis following infected-cell release from the colonic environment; Thompson & Wiberg, 1978; Asami *et al*., 1997). This study presents evidence that the LIN displayed by *imm*, *sp* and *5ts*1 mutants is truncated by a LO-like mechanism. Wild-type T4 phages thus manifest a *resistance* to both LO and LIN collapse (resistance to collapse) which I argue are important ecological functions of genes *imm* and *sp*.

2. Materials and methods

The backgrounds of the various phage strains employed are as follows. *5am*N135 is a mutant of T4D (Epstein *et al*., 1963) that was obtained via E. Kutter

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from the former G. Doermann collection and whose *5* gene has recently been sequenced (Takeda *et al.*, 1998). 37*am*N91 is a mutant of T4D (Epstein *et al.*, 1963) that was obtained from H. Bernstein. The 42*am*NG205 43*am*4306 double mutant were crossed by J. Delaney from laboratory strains of H. Bernstein. Both mutants were obtained from R. Edgar and are descendants of phage T4D.

The *sp* mutation is that of Emrich (1968). The *sp* gene of this mutant has also been recently sequenced and found to be a frame shift, one that replaces the last nine amino acids with a different six (T. Yonesaki, personal communication). The history of this mutant is somewhat complex and uncertain. It was originally isolated as a proflavine-induced revertant of a proflavine-induced *e* mutant, *e*J118 (Emrich, 1968), which itself was a triple mutant (with mutations in *e*, *ac* and *q*). Emrich cites Terzaghi *et al.* (1966) for this strain but, in fact, Terzaghi *et al.* did not explicitly employ strain *e*J118. Instead, they may have included the *e*J118 mutant among various unnamed mutants used in 'pairwise crosses between a number of closely linked, independently isolated, proflavine-induced mutants' since the two mutants explicitly described by Terzaghi *et al.* are apparently from the same series as *e*J118, i.e. *e*J42 and *e*J44. Terzaghi *et al.* (1966) explain why strain *e*J118 is a triple mutant: 'The markers *ac* and *q* enable phage to grow in the presence of otherwise inhibitory concentrations of acridine dyes and were present in all stocks to facilitate acridine mutagenesis'. However, they do not indicate whether their *ac q* strain of phage T4 was derived from T4D or T4B nor from where or from whom it or the individual markers were obtained.

To generate the presumptively single-*sp* mutant, the quadruple mutant was backcrossed to wild-type T4B (Emrich, 1968). Emrich does not explicitly indicate whether her backcrossed *sp* strain still possessed the *ac* or *q* mutations, though she does refer to subsequent constructs containing both the *sp* mutation and various *e* mutations as 'double mutant strains'. The Emrich strain was subsequently employed by Cornett (1974) who crossed this strain with the 42*am*NG205 mutant to generate *sp* 42*am*NG205. This double mutant was then backcrossed to T4D to generate an otherwise wild-type *sp* mutant. However, it is uncertain whether the strain used here is Emrich's single mutant or Cornett's further backcrossed single mutant. In either case, the *sp* strain employed here came from the H. Bernstein collection.

The *imm* mutant was originally found as a second mutation in a gene *39* mutant, *am*E142 (Mufti, 1972), which is a T4D-descendant from R. Edgar's collection (Vallée & Cornett, 1972). The *imm* mutant received the same treatment as Emrich's *sp* mutant in Cornett's (1974) hands and was similarly acquired for this study via H. Bernstein. The *imm sp* double mutant was also the product of Cornett and shares the *imm*- and *sp*-single-mutant histories.

The *5ts*1 mutant was originally selected as a pseudorevertant of the mutant *e*L5G and backcrossed to T4D, the wild-type T4 strain used by Kao & McClain (1980*a*). Kao & McClain do not indicate how the *5ts*1 mutant was mutationally generated. I obtained this strain from John Obringer. The *e*L5G strain, which contains a mutation deleting the majority of T4's tRNA genes, was obtained by Kao & McClain from J. (Emrich) Owen (Comer *et al.*, 1974). As with strain *e*J118, there is no indication whether *e*L5G was derived from T4B or T4D.

Crosses between 5*am*N135, 37*am*N91, *sp*, and/or *5ts*1 were done by myself. T4D is a wild-type T4 strain. *E. coli* S/6/5 (Su^-), unless otherwise noted, was used for experiments. *E. coli* CR63 (Su^+) was used for stock preparation. Described elsewhere are liquid culture preparation of phage stocks (Adams, 1959), Hershey broth growth medium (Steinberg & Edgar, 1962), M9 salts solution (Carlson & Miller, 1994) and anti-T4 serum (Abedon, 1992). Lysis profiles typically display significant variation between repetitions in terms of absolute turbidities attained and in terms of overall experimental durations. In addition, the extreme length of these assays, coupled with the fact that timings of lysis are typically only qualitatively predictable, inhibit the employment of consistent intervals between data points. Consequently, all experiments were done at least in triplicate but, unless otherwise indicated, only representative experiments are presented.

3. Results

(i) *T4* sp *mutant displays premature collapse*

Our knowledge that *sp* mutants are defective in LIN comes from observation of high-temperature plaque morphology and via lysis-profile assays (Emrich, 1968; Cornett, 1974; Kao & McClain, 1980*b*). Lysis-profile assays (Abedon, 1992) consist of following the turbidity of infected liquid cultures of *E. coli*, here using a Klett colorimeter (Klett Manufacturing, New York). Fig. 1 presents the lysis profiles at 37 °C of *E. coli* S/6/5 cultures infected with T4 phages mutated in gene *imm* (■) or gene *sp* (▲), mutations in both genes *imm* and *sp* (*imm sp*; ▼), the *5ts*1 mutation (◆) or no mutations (T4D, i.e. wild-type; ●). Lysis profiles of the type presented in Fig. 1 were also done at 29 °C and 42 °C, as discussed below, but are presented only in tabular form (Table 1).

At 37 °C, T4 *sp*-mutant-infected cells display LIN, though less than that displayed by T4D (see also Emrich, 1968; Cornett, 1974; Kao & McClain, 1980*b*). That is, *sp* mutants lyse prematurely under lysis-inhibiting conditions and thus display *premature collapse*. At 29 °C, the *sp* mutant displays a more wild-

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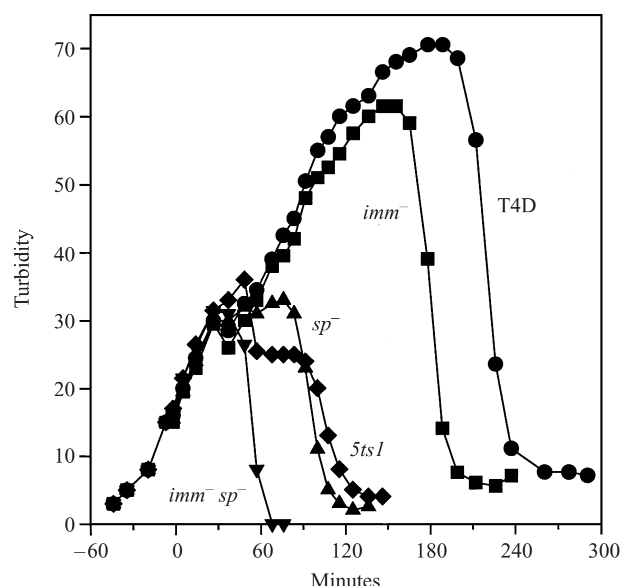

Fig. 1. Phage-mutant lysis profiles at 37 °C. Phages were added to bacterial suspensions when culture turbidity reached 15 (time zero; MOI 0·2). Note that culture turbidity initially rises because of replication of uninfected cells, but then stabilizes or declines for most phages after an initial period of infected-cell lysis (between approximately 30 and 50 min in this figure). Cultures (especially *imm* mutant and wild-type) then inexplicably undergo a period of turbidity rise (which presumably results from the infected cells becoming denser since (i) this turbidity rise is not associated with cell division (Abedon, 1992), (ii) plating for cell viable count following phage addition demonstrates no significant lack of phage infection (unpublished observation) and (iii) the turbidity rise is reversed upon LIN collapse (this figure)). This turbidity rise is then followed by a turbidity decline (LIN collapse) that defines the end of the lysis-inhibited latent period. Curves vary by phage genotype and include: (i) T4D (●), (ii) *imm* mutant (■), (iii) *sp* mutant (▲), (iv) *imm sp* double mutant (▼) and (v) *5ts*1 mutant (◆).

type LIN than that seen at 37 °C, but still much less than that displayed by T4D (Table 1). At 42 °C, *sp*-mutant cultures displayed little or no LIN (Table 1), a temperature sensitivity consistent with Emrich's (1968) observation of mutant plaque morphologies. Table 2 presents relative measurements of phage titres following LIN collapse. The *sp*-mutant LIN defect here too is particularly evident at higher temperatures. Overall, these results suggest that the wild-type gene *sp* contributes in some manner to the maintenance of the LIN state, perhaps particularly at higher temperatures, though alternatively the *sp* mutation may be temperature-sensitive.

(ii) *T4* imm *mutant displays premature collapse*

Unlike gene *sp*, the T4 *imm* gene has not been described as contributing to LIN. However, *imm sp* double-mutant-infected, lysis-inhibited cultures lyse sooner than *sp*-mutant cultures at both 29 °C and 37 °C (Fig. 1, Table 1). These results show that the *imm* gene can augment the *sp* gene's contribution to LIN. Consistently, lysis-inhibited cultures infected with the *imm* single mutant fail to sustain LIN for as long as wild-type-infected cultures (Fig. 1, Table 1). The *imm* mutation thus confers premature collapse. Cornett (1974), doing similar experiments (using *E. coli* BB at 37 °C), failed to note a shorter lysis-inhibited latent period for *imm*-infected cells as compared with wild-type. This can be explained by his apparently stopping experiments after 100 min and well before *imm* or wild-type collapse. Nevertheless, his figure 2 does show the *imm sp* double mutant lysing prior to the *sp* single mutant.

The turbidity of T4 *sp*-mutant cultures rises during LIN, while cultures infected with the *imm sp* double

Table 1. *Relative lysis-inhibited latent periods* (±*standard error*) *of mutant-infected cells*

Phage genotype	Normalized latent period length		
	29 °C	37 °C	42 °C
One-step latent period	0·15±0·01 (7)	0·14±0·01 (11)	0·15±0·01 (7)
Two-step latent period	0·30±0·01 (7)	0·29±0·02 (11)	0·31±0·01 (7)
Wild-type (*imm*$^+$ *sp*$^+$)	1·00±0·00 (7)	1·00±0·00 (11)	1·00±0·00 (7)
imm$^-$ mutant (*sp*$^+$)	0·71±0·01 (7)	0·76±0·03 (11)	0·87±0·03 (7)
*5ts*1 mutant (*imm*$^+$ *sp*$^+$)	0·41±0·03 (3)	0·47±0·02 (3)	No data
sp$^-$ mutant (*imm*$^+$)	0·61±0·03 (7)	0·42±0·02 (11)	0·28±0·02 (7)
imm$^-$ *sp*$^-$ double	0·32±0·04 (6)	0·25±0·02 (11)	0·24±0·02 (7)

The time of lysis was defined as the point at which a culture displayed less turbidity than it did at the point of phage addition (e.g. turbidity at time = 0 in Fig. 1). All times are presented normalized to that seen with the T4D wild-type. The *5ts*1 mutant was not used at high temperatures. The one-step latent period was defined from experience as being approximately 30 min at 37 °C and 42 °C, and approximately 50 min at 29 °C (times associated with the tail-ends of rise periods rather than the first moments of lysis). The two-step latent periods are simply double the calculated one-step latent periods. Since these lysis profiles were initiated with a low phage multiplicity (0·1–0·2), a typical curve will display two lytic cycles and hence should be, *in the absence of LIN*, approximately two steps long. The number of experiments done is shown in parentheses.

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Table 2. *Relative burst titres and burst sizes* (±*standard error*) *of* imm- *and* sp-*mutant infected cells*

Phage genotype	Normalized lysis-inhibition burst titre 29 °C	37 °C	42 °C	Single-step burst 37 °C
Wild-type (*imm*$^+$ *sp*$^+$)	1·00 (3)	1·00 (7)	1·00 (3)	1·00 (4)
imm$^-$ mutant (*sp*$^+$)	0·87±0·06	1·00±0·03	1·06±0·07	1·08±0·14
sp$^-$ mutant (*imm*$^+$)	0·59±0·06	0·31±0·06	0·06±0·02	1·08±0·14
imm$^-$ *sp*$^-$ double mutant	0·13±0·06	0·12±0·02	0·04±0·01	1·05±0·11

LIN burst titre is the phage titre following the lysis of lysis-inhibited cultures. Single-step burst size, a 'true' burst-size measurement (done at 37 °C), is equal to: (final phage titre following low-cell-density lysis)/(initial infective-centre titre following high-cell-density phage adsorption). Average burst size for these experiments was approximately 130. Burst titres (or, for the fourth column, burst sizes) were determined in parallel experiments (i.e. one column of values per experiment). The number of parallel experiments done, per column, is presented in parentheses. For each parallel experiment, relative burst titres were found by dividing individual burst titres by the T4D burst titre.

mutant do not (Fig. 1). The absence of a turbidity rise at 37 °C with the double mutant, but not with the *sp* single mutant, is an additional indication that Imm plays some role in LIN. Similarly, at both 29 °C and 37 °C, *sp*-mutant-infected cultures display a larger post-lysis titre (burst titre) than do cultures infected with the *imm sp* double mutant (Table 2). In so far as burst titre is a measure of LIN, this result is further corroboration that gene *imm* plays some role in LIN. However, one must be cautious in interpreting burst titres. For example, the number of phages remaining following LIN collapse is presumably affected not just by the duration of infection, but also by variation in the increase in burst size over time (e.g. perhaps burst titres slow in their rate of increase as infections age; Wang *et al*., 1996) and the degree to which progeny phages adsorb to infected cells. Thus, the finding that the *imm* single mutant's burst titre was nearly identical to that of wild-type, at all three temperatures (Table 2), does not necessarily imply a contradiction with the lysis profile data presented in Fig. 1. Consequently, a reasonable interpretation of the data, *in toto*, is that the *imm* mutant displays a short LIN latent period, and that this LIN defect is manifest regardless of which allele is found at the *sp* locus.

(iii) *Single-step growth*

In contrast to the lysis-inhibited burst-titre results, *imm*, *sp*, *imm sp* and wild-type phages grown under single-step growth conditions (Carlson, 1994) show similar burst sizes, averaging about 130 progeny phages produced per infected cell under the conditions employed (Table 2). These phages also display similar single-step latent periods (single-step growth curves not presented and only 37 °C experiments done). Whatever is responsible for the display of premature collapse by *imm* and *sp* mutants under the conditions employed, it apparently does not significantly affect phage growth, so long as secondary adsorptions are absent.

(iv) *Secondary adsorptions contribute to* imm *and* sp *premature collapse*

The lysis of T4-infected cells could occur as a consequence of mechanisms of either LI or LO, but standard lysis profile assays cannot distinguish these mechanisms, at least so long as no effort is made to prevent secondary adsorptions. Note, however, that secondary adsorptions are required for the induction of LIN. It is therefore impossible to observe wild-type LIN absent secondary adsorptions. This requirement for secondary adsorptions complicates both experimental design and interpretation when attempting to distinguish the effects of LI and LO on LIN collapse, especially when the mutants being characterized are sensitive to LO. Nevertheless, at least under some conditions, a strategic application of anti-T4 serum following LIN-inducing secondary adsorption can result in a dramatic extension of mutant LIN, a result consistent with the existence of a LO-like mechanism of premature collapse.

Addition of inactivating antiserum (rabbit, anti-phage T4) to wild-type cultures after LIN induction inhibits LIN collapse (Abedon, 1992). Fig. 2 shows the effect of anti-T4 serum addition to a 29 °C *imm*-mutant culture. Note that with anti-T4 serum addition the *imm*-mutant's collapse (□) is significantly delayed relative to the timing of collapse displayed by the *imm* mutant without anti-T4 serum (■), and comes to approach that displayed by that of wild-type, also without anti-T4 addition (●). Nearly identical results were seen using 29 °C *sp* and 37 °C *imm* mutant cultures (data not presented). By contrast, the *sp* mutant's collapse at 37 °C was not inhibited by anti-T4 serum under the conditions employed (not shown). I speculate, but lack evidence to prove, that this latter result is a consequence of the *sp* mutant displaying sufficient susceptibility to LO at this temperature that the secondary phages added to induce LIN also induce LO despite the subsequent addition of anti-T4 serum. Though not necessarily the case with the *sp*

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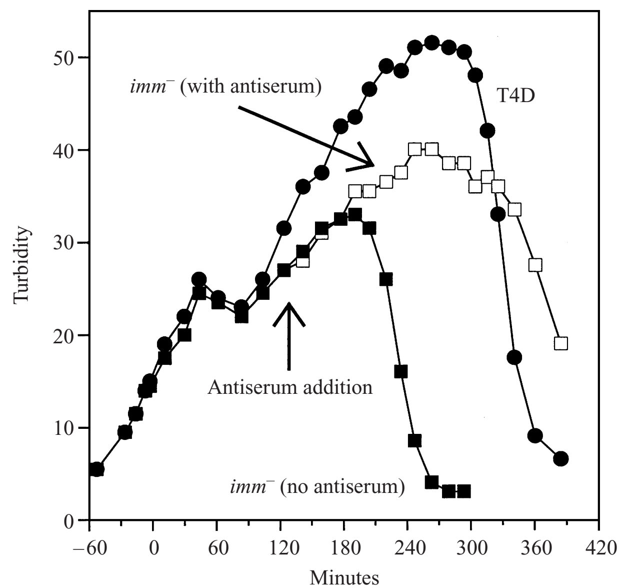

Fig. 2. Lysis profiles of *imm* mutant (29 °C) with or without anti-T4 serum addition. The protocol was the same as that employed for Fig. 1, except that a single *imm* culture was divided into two cultures a few minutes before anti-T4 serum addition (denoted by arrow). Symbols: (i) T4D (●), (ii) *imm*, no anti-T4 serum (■) and (iii) *imm* plus anti-T4 serum (□).

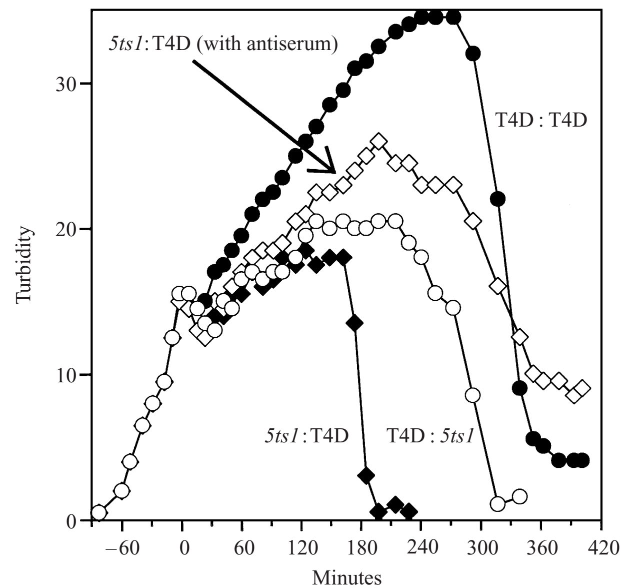

Fig. 3. Lysis profiles of T4D and *5ts*1 mutant phages following various combinations of explicitly added secondary phages and anti-T4 serum addition. Experimental protocol is as described in the text. Symbols: (i) T4D primary, T4D secondary, no anti-T4 serum (●); (ii) T4D primary, *5ts*1 secondary, no anti-T4 serum (○); (iii) *5ts*1 primary, T4D secondary, no anti-T4 serum (◆); and (iv) *5ts*1 primary, T4D secondary, anti-T4 serum (◇).

mutant at 37 °C, I nevertheless infer from these experiments that in the absence of anti-T4 serum, secondary adsorptions contribute to the induction of *imm*-mutant (and 29 °C *sp*-mutant) premature collapse. In other words, and with the above caveat, it appears that at least some of the conditions that would be expected to inhibit LO can also inhibit premature collapse.

(v) *Secondary adsorptions contribute to* 5ts*1 premature collapse*

As with the *imm* and *sp* mutants, the T4 *5ts*1 mutant displays premature collapse (Kao & McClain, 1980*b* and Fig. 1). However, unlike in the *imm*-mutant and 29 °C *sp*-mutant experiments presented in Fig. 2, *5ts*1 premature collapse was only partially inhibited by anti-T4 serum at 37 °C. Contrasting with the temperature sensitivity of the *5ts*1 mutation, even less inhibition by anti-T4 serum was observed at 29 °C (curves not presented). A possible explanation for these results, and for the comparable 37 °C *sp*-mutant result presented above, is that secondary adsorptions, normally required to fully induce LIN in wild-type-infected cells, may at the same time have a large negative effect on LIN. Again, the effects of secondary adsorptions are expected to be especially negative when employing phage mutants that are sensitive to LO, and these effects may be sufficiently negative that blockage of later secondary adsorptions via anti-T4 serum fails to significantly influence the course of premature collapse.

With *5ts*1 mutants, however, it is possible to bypass this technical difficulty by reducing the lytic effect of secondary adsorptions. This is possible because, unlike *imm*- and *sp*-mutant-infected cells (which are simply more susceptible to the lytic effects of secondary adsorptions than are wild-type-infected cells), *5ts*1 mutants display their secondary adsorption-associated, enhanced lytic effect only upon the adsorption of *5ts*1 free phages to otherwise wild-type-infected cells. Substituting wild-type secondary adsorption for *5ts*1 secondary adsorption should therefore have a positive effect on LIN.

This hypothesis was tested using a temperature of 29 °C and infections initiated with *5ts*1 or T4D (wild-type) phage (MOI 5). Twenty-five minutes later a secondary phage (*5ts*1 or wild-type) was added to induce LIN (the multiplicity of this secondary adsorption was 10). As was expected given that *5ts*1 phage particles are especially lytic, T4 wild-type-infected cells displayed more LIN (Fig. 3) following secondary adsorption by wild-type phage (●) than following secondary adsorption by *5ts*1-mutant phage (○). Note, however, that *5ts*1-infected cells show premature collapse even given explicit secondary adsorption by T4D (◆). Thus, the observed premature collapse could be due to the action of intracellular *5ts*1 lysozyme, or could be a result of LO induced by the *5ts*1-phage progeny released from *5ts*1-infected

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cells over the course of the experiment (Abedon, 1992). Were the latter explanation correct, then addition of anti-T4 serum to *5ts*1 cultures (after secondary adsorption with T4D) should further inhibit premature collapse. I added anti-T4 serum 10 min after explicit secondary adsorption. Cells that had a primary *5ts*1 infection, T4D secondary adsorption and anti-T4 serum treatment (◇) had a latent period approaching that of untreated, wild-type-infected cells (i.e. T4D primary and secondary phage; ●). Since the *5ts*1 mutant is thought to act by bypassing normal Sp function (Kao & McClain, 1980*a*, *b*), these results serve to corroborate evidence, presented above, that secondary adsorptions contribute to the premature collapse seen with *sp* mutants. In other words, these data are consistent with wild-type gene *sp* resistance to premature collapse occurring via the inhibition of the LO-inducing action of the gp*5* associated with secondary phages, rather than via the inhibition of a LI-like mechanism.

(vi) *Premature collapse without auto-secondary adsorption*

The experiments presented above suggest that premature collapse, exhibited by *imm*, *sp* and *5ts*1 mutants, is a consequence of secondary adsorption. For experiments presented in this section, LIN was observed under more controlled conditions than can be accomplished using lysis-profile assays. In addition, experiments were monitored as a function of phage progeny release rather than turbidity decline. *E. coli* S/6/5 cells were infected with a low multiplicity ($\ll 1$) of non-amber T4 mutants (wild-type, *imm*, *sp*, *imm sp* and *5ts*1), diluted to a concentration of $\sim 10^4$ cells/ml, and subsequently adsorbed with relatively high concentrations of 42*am*NG205 43*am*4306 double amber-mutant phage ($\sim 5\times10^8$/ml final concentration). Under these conditions, secondary adsorption should occur at a low but nearly constant rate (Abedon, 1990).

This protocol keeps the genotype of primary and secondary phages distinct. For example, in this assay cells infected by *5ts*1 primary phages are subjected to secondary adsorption only by wild-type gene *5* phages. This 'selective' adsorption occurs because infected cells are diluted too far for the progeny phages released by infected cells to make up a significant fraction of secondary phages. Similarly, cells are sufficiently dilute that minimal progeny phages are lost to secondary adsorption. Consequently, *5ts*1 primary-infected cells may prematurely collapse only if *5ts*1 lysozyme acts intracellularly to truncate LIN. This is true since (i) secondary phages carry a wild-type gene *5* allele and (ii) the *5ts*1 primary phage presumably carries a wild-type *sp* allele (e.g. Fig. 3 and Kao & McClain, 1980*a*). On the other hand, LIN

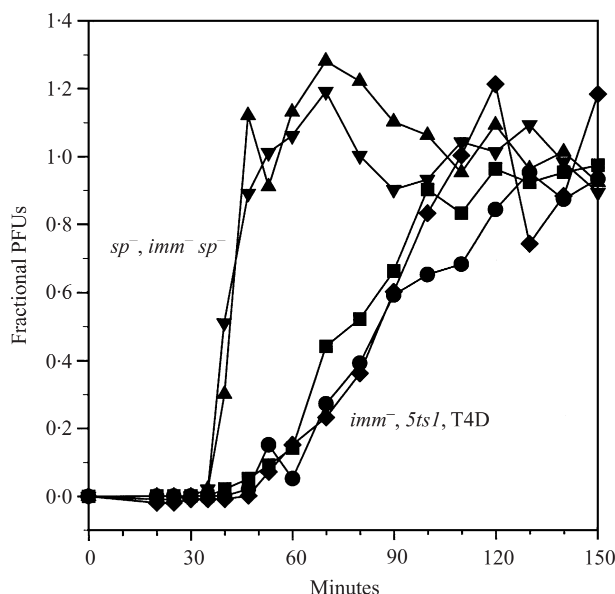

Fig. 4. Assay for LIN collapse at low cell density. Primary phages (those phages that initiate infections) were adsorbed for 5 min to 10^9/ml cells washed in M9 salts solution at MOI 0·1, then diluted 10^5-fold into Hershey broth (time = 0) to initiate infected-cell metabolism and give a final cell concentration of 10^4 cells/ml. Ten minutes after this initiation of metabolism, secondary phages were added to a final concentration of 5×10^8/ml (this concentration gives a calculated rate of adsorption of roughly one phage per cell per minute, which corresponds to a total loss of only about 0·002% of the free phages per minute; Stent, 1963; Abedon, 1990). An 42*am*NG205 43*am*4306 double mutant secondary phage was used so that secondary phages would be unable to produce progeny after adsorbing, and so amber-revertant progeny phages would be produced at an extremely low rate. Non-amber plaque-forming units (PFUs) were determined using *E. coli* S/6/5 indicator bacteria and the soft-agar-overlay method (Adams, 1959). 'Fractional PFUs' were found by dividing each data point (PFUs) by the average number of PFUs (= infected bacteria + unadsorbed, non-amber phages) present prior to the addition of secondary phages, subtracting 1 (which adjusts PFUs to zero), and then dividing by the mean number of PFUs calculated as above (determined between 130 to 185 min after infection, thus setting the post-lysis mean PFUs to 1). For clarity, only the first 150 min of each experiment is shown: (i) T4D (●), (ii) *imm* mutant (■), (iii) *sp* mutant (▲), (iv) *imm sp* double mutant (▼) and (v) *5ts*1 mutant (◆).

induced in cells infected by a *sp*-mutant primary phage should prematurely collapse because of either intracellular or extracellular gp*5* action depending, of course, on which occurs.

If extracellular gp*5* induces premature collapse (i.e. that associated with secondary phages), then upon secondary adsorption by gp*5* wild-type phages, the *5ts*1 mutant should have lysis kinetics resembling those of T4D. Alternatively, if intracellular 5 induces premature collapse, then the *5ts*1 mutant should have lysis kinetics resembling those of the *sp* mutant. As presented in Fig. 4, the lysis of the *5ts*1 mutant (◆)

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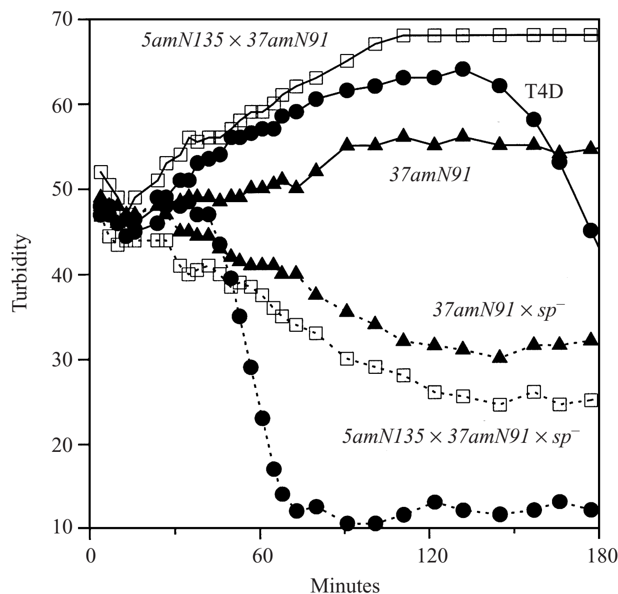

Fig. 5. Lysis profiles of lysis-inhibited cultures displaying a truncated gene 5. Cultures were grown at 37 °C with bubbling to a concentration of 10^8 cells/ml, washed, and then resuspended to a concentration of 10^9 cells/ml in M9 salts solution. Phage adsorption (MOI 5) was accomplished at this concentration on a vertical vibrator (Arthur H. Thomas, Philadelphia, PA) for 20 min. Metabolism was initiated by adding an equal volume of 2 × broth, and then sufficient broth to bring the cell concentration to 2×10^8/ml. The cell/phage mixture at this point was placed in nephelo flasks in a gyrotary-shaker water bath. Curves with dotted lines are *sp* mutants (*sp*, ●; *sp* 37*am*N91, ▲; *sp* 5*am*N135 37*am*N91, □). Continuous lines are wild-type for *sp* (T4D, ●; 37*am*N91, ▲; 5*am*N135 37*am*N91, □). Filled symbols refer to phage strains containing the wild-type *5* allele (T4D and *sp*, ●; 37*am*N91 and *sp* 37*am*N91, ▲). Open symbols carry the 5*am*N135 mutant (5*am*N135 37*am*N91 and *sp* 5*am*N135 37*am*N91, □). A *sp* 5*am*N135 37*am*N91 triple mutant was added to all cultures as the secondary phage, at MOI 5, 20 min after the start of primary-phage metabolism.

was nearly identical to that of T4D (●) and was clearly distinguishable from that of the *sp* (▲). The figure also shows that this protocol is apparently unable to distinguish the mutant *imm* allele from the wild-type *imm* allele (■ vs ● and ▼ vs ▲). In conclusion, under the conditions of this experiment it is extracellular, not intracellular gp*5* that induces premature collapse, that is, by a mechanism better resembling LO than LI.

(vii) *Premature collapse without intracellular* gp*5*

Phages having a non-functional gene *5* allele are incapable of inducing 5-associated LI (Kao & McClain, 1980*b*). If a *sp* mutant displays premature collapse due to the action of intracellular gp*5*, then a *sp* 5*am*N135 double mutant, infecting a host lacking an amber suppressor (*E. coli* S/6/5; MOI 5), should manifest untruncated LIN. Here I compare the lysis kinetics of wild-type (T4D) and five mutant T4 strains. Free phages lacking 5 protein are known to be adsorption deficient (Kao & McClain, 1980*a*; Nakagawa *et al.*, 1985). Similarly, 37*am*N91 phage, when grown on amber non-suppressing hosts, produce progeny that possess incomplete long tail fibres and that consequently are unable to initiate the adsorption process (e.g. 37*am*N91 phage show LIN only given explicit secondary-phage addition; Paddison *et al.*, 1998). T4 37*am*N91 mutants were thus employed as wild-type 5, adsorption-incompetent controls, and for consistency the 37*am*N91 mutation was incorporated into the 5*am* N135 mutants as well.

The results of this experiment, as presented in Fig. 5, strongly suggest that intracellular 5 plays little or no role in the induction of premature collapse in *sp* mutants. Particularly, *sp* 5*am*N135*am*N91 and *sp* 37*am*N91 mutants (□ and ▲, respectively, both dotted lines) exhibit similar lysis profiles. The absent LIN turbidity rise observed with the adsorption-defective *sp*-mutant-infected cells is presumably a consequence of damage incurred upon the explicit secondary adsorption employed to induce LIN. Again, a LO-like mechanism of premature collapse is more consistent with the data than a LI-like mechanism.

4. Discussion

(i) *Ecology of resistance to collapse*

The ecological explanation for why T4 bacteriophages display LIN is two-fold (Abedon, 1990):

First, T4-infected cells kill adsorbing T4 phages via a mechanism termed superinfection exclusion (Abedon, 1994). By displaying LIN, T4-infected cells avoid lysis, thereby delaying exposure of progeny phages to other, not-yet-lysed, T4-infected cells.

Secondly, with an extended latent period comes an increased burst size. This burst-size increase may be especially beneficial when uninfected cells are scarce (Abedon, 1990). However, in environments flush with uninfected cells, extended latent periods can interfere with the rapid establishment of multiple, parallel infections, and could significantly slow phage population growth (Abedon, 1989; Wang *et al.*, 1996; and references cited therein). Secondary adsorption and LIN induction thus serve phage-infected cells as a means of distinguishing environments that contain high concentrations of infected cells from those that contain high concentrations of *un*infected cells (Abedon, 1990).

(ii) *Resistance to collapse is resistance to LO*

Rapid lysis mutants, which are unable to inhibit their lysis given secondary adsorption, are at a significant disadvantage when competing against wild-type phages in unstructured (i.e. well-mixed) environments

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such as in broth culture (Hershey, 1946*a*). Mutants that display more LIN than rapid-lysis mutants, but less than wild-type, are presumably also disadvantaged when competing against wild-type phages in unstructured environments, though perhaps less disadvantaged than true rapid-lysis mutants. In both cases, the disadvantage results from an exhibition of a smaller burst size and an increased exposure of progeny phages to not-yet-lysed infected cells (Abedon, 1990). Thus, T4 genes *imm* and *sp* presumably can contribute to the fitness of T4 phages by extending the LIN latent period.

T4 resistance to LIN collapse mechanistically resembles T4 resistance to LO. Evidence for this assertion includes: (i) wild-type, population-wide LIN collapse is propagated (and may even be induced) by a mechanism associated with secondary adsorption (Abedon, 1992); (ii) both *imm* and *sp* mutants are more sensitive than wild-type to LO (reviewed in Abedon, 1994); (iii) inhibiting secondary adsorption extends the duration of mutant *imm*, *sp* and *5ts*1 LIN (Figs 2–4); (iv) replacing *5ts*1 secondary adsorption with less lytic, wild-type secondary adsorptions extends the duration of LIN (Figs 3, 4); (v) mutant *sp* LIN is truncated even when the intracellular gp*5* is not functional (Fig. 5); (vi) finally, the 5 protein is synthesized in an inactive state, may not attain lysozyme activity until it is incorporated into the phage baseplate, and appears to have low activity following baseplate incorporation but prior to the conformational change normally seen upon adsorption (Nakagawa *et al*., 1985; Mosig *et al*., 1989). These latter points argue that 5 lysozyme is inefficiently configured for passage from the cytoplasm through the inner membrane, which the 5 protein would have to do to digest the murein layer of infected cells undergoing LIN. It is likely, therefore, that premature collapse occurs primarily because of LO-like, secondary-adsorption-induced damages.

By contrast, Kao & McClain (1980*b*) proposed that intracellular 5 induces the premature collapse seen with *sp* or *5ts*1 mutants, but this speculation is supported solely by a lysis profile assay. Lysis profile assays cannot distinguish between LI-like and LO-like mechanisms. Nevertheless, while the arguments and experiments presented here serve to contradict Kao & McClain's speculation on the role of intracellular gp*5* in truncating LIN, they simultaneously reaffirm the significance of Kao & McClain's pivotal contributions to our understanding of the roles of gp*5* and gp*sp* in LO and resistance to LO. Similarly, it is likely that the LI phenotype observed with *e sp* double mutants is, in fact, only minimally relevant to our understanding of wild-type lysis and LIN, despite the role this mutant phenotype played in both the discovery and subsequent characterization of gene *sp* (Emrich, 1968; Kao & McClain, 1980*a*, *b*).

(iii) *Mechanism of premature collapse*

What follows is my working scenario (derived in Abedon, 1992; see also Abedon, 1994) for the role played by LO in wild-type LIN collapse. Initial secondary adsorptions induce LIN. These secondary adsorptions also inflict some amount of cell-envelope damage, despite the presence of wild-type *imm* and *sp* alleles. This damage may play a role especially in the timing of the initiation of LIN collapse. During the subsequent course of LIN, some infected cells spontaneously lyse, releasing additional free phages into the environment. Additional secondary adsorption thus occurs. At some point, secondary adsorptions sufficiently damage the cell envelope that spontaneous lytic events become more numerous. Lysis results in progeny-phage release, increasing the adsorption load on infected cells. Additional lysis results, this positive feedback resulting in LIN collapse.

In cultures infected with *imm*- or *sp*-mutant phages, a similar series of events presumably occurs. Because *imm* and *sp* mutants are more susceptible to secondary adsorption-induced damage, however, the sequence is accelerated, resulting in premature collapse. With *5ts*1 lysis-inhibited cultures, the same sequence also probably occurs, only here it is not infected cells that are more susceptible to secondary adsorption-induced damage but adsorbing secondary phages that are more lytic.

The degree to which protection against the damaging effects of secondary adsorptions is relevant to successful phage growth is probably dependent on secondary-phage multiplicity. If all other factors are held constant, effective (i.e. time-dependent) phage multiplicities should be higher at higher host-cell densities simply because free phages need travel shorter distances before colliding with infected cells (Abedon, 1990). As with LIN itself (Abedon, 1989, 1990, 1992, 1994), and for the same reason, resistance to collapse consequently may be an adaptation particularly to phage growth in the presence of higher cell densities.

(iv) *Simplification of our understanding of the LIN phenotype*

This resistance-to-LO model of resistance to LIN collapse serves to unite two of the phenotypes associated with *imm*, *sp* and *5ts*1 mutants as a single mechanism, i.e. premature truncation of LIN and sensitivity to LO. This synthesis is similar in spirit to the recently proposed uniting of the two primary T4 *rII*-mutant phenotypes – restriction by phage lambda lysogens and rapid lysis – into a single mechanism (Paddison *et al*., 1998). The actual induction of LIN must involve some secondary adsorption signal that turns off the normal mechanism

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of T4 LI (Paddison *et al*., 1998). How this induction occurs continues to defy facile explanation (Mathews, 1994; Alberts, 1994; Paddison *et al*., 1998). This confusion, in part, is a consequence of the large number of phage loci apparently required for the occurrence of wild-type LIN (Abedon, 1994). The mechanisms proposed for the roles played by genes *imm*, *sp*, *rIIA* and *rIIB* in wild-type LIN, however, suggest that these loci are not directly responsible for the induction of the LIN phenotype, i.e. the propagation of the secondary adsorption signal. Removal of these four loci from consideration could significantly simplify the elucidation of the mechanism of LIN induction.

(v) *Resistance to collapse and superinfection exclusion*

Genes *imm* and *sp* are also involved in superinfection exclusion. As argued by Obringer (1988), superinfection exclusion may have evolved to protect the infected cell resource from costs associated with subsequent (super)infection, particularly as a mechanism of genetic exclusion (see Abedon, 1994). As discussed throughout this study, genes *imm* and *sp* may be adaptations necessary for wild-type LIN – and this is especially true for gene *sp*, which appears to play a larger role than gene *imm* in LIN (Fig. 1; Tables 1, 2) and a smaller role in superinfection exclusion (Obringer, 1988; Abedon, 1994). We can therefore infer that T4 superinfection exclusion evolved exclusively as a mechanism of genetic exclusion only so long as gene *imm*- and *sp*-associated superinfection exclusion and resistance to premature collapse differ mechanistically. However, no such differences are known (Abedon, 1994). Consequently, I propose that the advantages associated with the superinfection-exclusion phenotype are at best co-responsible for the evolution of the *imm* and *sp* pleiotropy. Taking this interpretation to an extreme, it is conceivable that T4 superinfection exclusion is simply an advantageous by-product of protective mechanisms put in place primarily as a means of extending the T4 lysis-inhibited latent period.

(vi) *Conclusion*

LIN appears to play an important role in the wild-type lytic cycle of T4 phages (Abedon, 1990). However, it is unknown to what extent the exhibition of LIN may contribute to the cell-killing efficacy hypothesized to be a necessary component of phage-based antibacterial therapies. Here I have presented an explanation for the role of three T4 loci – *imm*, *sp* and *5* – in the maintenance and reversal of the lysis-inhibited state. Furthermore, I have characterized and compared the degree of LIN displayed by the *imm*, *sp* and *5ts*1 mutants. The existence of their intermediate displays of LIN should allow increased versatility in future explorations of the utility of LIN *in situ*, as well as a longer-term potential for fine-tuning the display of LIN during phage therapeutics.

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References

Abedon, S. T. (1989). Selection for bacteriophage latent period length by bacterial density: a theoretical examination. *Microbial Ecology* **18**, 79–88.

Abedon, S. T. (1990). Selection for lysis inhibition in bacteriophage. *Journal of Theoretical Biology* **146**, 501–511.

Abedon, S. T. (1992). Lysis of lysis inhibited bacteriophage T4 infected cells. *Journal of Bacteriology* **174**, 8073–8080.

Abedon, S. T. (1994). Lysis and the interaction between free phages and infected cells. In *The Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 397–405. Washington, DC: ASM Press.

Ackermann, H.-W. & Dubow, M. S. (1987). *Viruses of Prokaryotes*, vol. 1, *General Properties of Bacteriophages*. Boca Raton, Florida: CRC Press.

Adams, M. H. (1959). *Bacteriophages*. New York: Interscience.

Alberts, B. M. (1994). Epilogue: unsolved mysteries and the T4 paradigm. In *Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 487–488. Washington, DC: ASM Press.

Alisky, J., Iczkowski, K., Rapoport, A. & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *Journal of Infection* **36**, 5–15.

Asami, K., Xing, X. H., Tanji, Y. & Unno, H. (1997). Synchronized disruption of *Escherichia coli* cells by T4 phage infection. *Journal of Fermentation and Bioengineering* **83**, 511–516.

Barrow, P. A. & Soothill, J. S. (1997). Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. *Trends in Genetics* **5**, 268–271.

Bode, W. (1967). Lysis inhibition in *Escherichia coli* infected with bacteriophage T4. *Journal of Virology* **1**, 948–955.

Carlson, K. (1994). Single-step growth. In *Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 434–437. Washington, DC: ASM Press.

Carlson, K. & Miller, E. S. (1994). Working with T4. In *Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 421–426. Washington, DC: ASM Press.

Comer, M. M., Guthrie, C. & McClain, W. H. (1974). An ochre suppressor of bacteriophage T4 that is associated with a transfer RNA. *Journal of Molecular Biology* **90**, 665–676.

Coombs, D. H. & Arisaka, F. (1994). T4 tail structure and function. In *The Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 259–281. Washington, DC: ASM Press.

Cornett, J. B. (1974). Spackle and immunity functions of bacteriophage T4. *Journal of Virology* **13**, 312–321.

Demerec, M. & Fano, U. (1945). Bacteriophage-resistant mutants in *Escherichia coli*. *Genetics* **30**, 119–136.

https://doi.org/10.1017/S0016672399003833 Published online by Cambridge University Press

Doermann, A. H. (1948). Lysis and lysis inhibition with *Escherichia coli* bacteriophage. *Journal of Bacteriology* **55**, 257–275.

Emrich, J. (1968). Lysis of T4-infected bacteria in the absence of lysozyme. *Virology* **35**, 158–165.

Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H. & Lielausis, A. (1963). Physiological studies of conditional lethal mutants of bacteriophage T4D. *Cold Spring Harbor Symposia on Quantitative Biology* **28**, 375–394.

Goldberg, E., Grinius, L. & Letellier, L. (1994). Recognition, attachment, and injection. In *The Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 347–356. Washington, DC: ASM Press.

Hershey, A. D. (1946*a*). Mutation of bacteriophage with respect to type of plaque. *Genetics* **31**, 620–640.

Hershey, A. D. (1946*b*). Spontaneous mutations in bacterial viruses. *Cold Spring Harbor Symposia on Quantitative Biology* **11**, 67–77.

Kaiser, D. (1996). Bacteria also vote. *Science* **272**, 1598–1599.

Kao, S. H. & McClain, W. H. (1980*a*). Baseplate protein of bacteriophage T4 with both structural and lytic functions. *Journal of Virology* **34**, 95–103.

Kao, S. H. & McClain, W. H. (1980*b*). Roles of T4 gene *5* and gene *s* products in cell lysis. *Journal of Virology* **34**, 104–107.

Kutter, E. (1998). Phage therapy: bacteriophages as antibiotics. At http://www.evergreen.edu/user/T4/PhageTherapy/phagethea.html.

Lederberg, J. (1996). Smaller fleas… ad infinitum: therapeutic bacteriophage redux. *Proceedings of the National Academy of Sciences of the USA* **93**, 3167–3168.

Levin, B. R. & Bull, J. J. (1996). Phage therapy revisited: the population biology of a bacterial infection and its treatment with bacteriophage and antibiotics. *American Naturalist* **147**, 881–898.

Lu, M. & Henning, U. (1989). The immunity (*imm*) gene of *Escherichia coli* bacteriophage T4. *Journal of Virology* **63**, 3472–3478.

Lu, M.-J., Stierhof, Y.-D. & Henning, U. (1993). Location and unusual membrane topology of the immunity protein of the *Escherichia coli* phage T4. *Journal of Virology* **67**, 4905–4913.

Mathews, C. K. (1994). An overview of the T4 developmental program. In *Molecular Biology of Bacteriophage T4* (ed. J. D. Karam), pp. 1–8. Washington, DC: ASM Press.

Mosig, G., Lin, G. W., Franklin, J. & Fan, W. H. (1989). Functional relationships and structural determinants of two bacteriophage T4 lysozymes: a soluble gene *e* and a baseplate-associated gene *5* protein. *New Biologist* **1**, 171–180.

Mufti, S. (1972). A bacteriophage T4 mutant defective in protection against superinfecting phage. *Journal of General Virology* **17**, 119–123.

Nakagawa, H., Arisaka, F. & Ishii, S.-I. (1985). Isolation and characterization of the bacteriophage T4 tail-associated lysozyme. *Journal of Virology* **54**, 460–466.

Obringer, J. W. (1988). The functions of the phage T4 immunity and spackle genes in genetic exclusion. *Genetical Research* **52**, 81–90.

Paddison, P., Abedon, S. T., Dressman, H., Gailbreath, K., Mosser, E., Neitzel, J., Guttman, B. & Kutter, E. (1998). Lysis inhibition and fine-structure genetics in bacteriophage T4. *Genetics* **148**, 1539–1550.

Rutberg, B. & Rutberg, L. (1965). Role of superinfecting phage in lysis inhibition with phage T4 in *Escherichia coli*. *Journal of Bacteriology* **90**, 891–894.

Steinberg, C. M. & Edgar, R. S. (1962). A critical test of a current theory of recombination in bacteriophage. *Genetics* **47**, 187–208.

Stent, G. (1963). *Molecular Biology of Bacterial Viruses*. San Francisco: WH Freeman.

Takeda, S., Hoshida, K. & Arisaka, F. (1998). Mapping of functional sites on the primary structure of the tail lysozyme of bacteriophage T4 by mutation analysis. *Biochimica et Biophysica Acta* **1384**, 243–252.

Tarahovsky, Y. S., Ivanitsky, G. R. & Khusainov, A. A. (1994). Lysis of *Escherichia coli* cells induced by bacteriophage T4. *FEMS Microbiology Letters* **122**, 195–199.

Terzaghi, E., Okada, Y., Streisinger, G., Emrich, J., Inouye, M. & Tsugita, A. (1966). Change of a sequence of amino acids in phage T4 lysozyme by acridine-induced mutations. *Proceedings of the National Academy of Sciences of the USA* **56**, 500–507.

Thompson, S. & Wiberg, J. S. (1978). Late effect of bacteriophage T4D on the permeability barrier of *Escherichia coli*. *Journal of Virology* **25**, 491–499.

Vallée, M. & Cornett, J. B. (1972). A new gene of bacteriophage T4 determining immunity against superinfecting ghosts and phage in T4-infected *Escherichia coli*. *Virology* **48**, 777–784.

Wang, I.-N., Dykhuizen, D. E. & Slobodkin, L. B. (1996). The evolution of phage lysis timing. *Evolutionary Ecology* **10**, 545–558.

Young, R. (1992). Bacteriophage lysis: mechanisms and regulation. *Microbiological Reviews* **56**, 430–481.

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