CHARACTERIZATION OF T4 MUTANTS THAT PARTIALLY SUPPRESS THE INABILITY OF T4*rII* TO GROW IN LAMBDA LYSOGENS

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ABSTRACT

In the course of isolating viable T4 deletions that affect plaque morphology (HOMYK and WEIL 1974), two closely linked point mutants, *sip1* and *sip2*, were obtained. They map between genes *t* and 52, cause a reduction in plaque size and burst size, and partially suppress the lethality of *rII* mutants for growth in lambda lysogens. These characteristics demonstrate that *sip1* and *sip2* are similar to mutants previously reported by FREEDMAN and BRENNER (1972). In addition, D. HALL (personal communication) has shown that *sip1* and *sip2* are similar to the mutant *farP85*, which affects the regulation of a number of early genes (CHACE and HALL 1975).——*Sip* suppression of *rII* mutants can be demonstrated in one-step growth experiments, even when both *rII* genes are completely deleted. This indicates that *sip* mutants do not simply reduce the level of *rII* gene products required for growth in a lambda lysogen. Instead, they alter the growth cycle so as to partially circumvent the need for any *rII* products.——Mutations at two other sites, designated L_1 and L_2, reverse the poor phage growth caused by *sip* and, in the one case tested, reverse the *rII*-suppressing ability of *sip*.

TWO effects of *rII* mutations in T2 and T4 have been recognized for a long time. First, such mutations prevent superinfection lysis inhibition (DOERMANN 1948), thus giving rise to the characteristic "rapid lysis" or "r" type plaque (HERSHEY 1946). Second, such mutants are unable to grow on hosts lysogenic for lambda *rex*$^+$ (BENZER 1955; HOWARD 1967). BENZER (1955) recognized the experimental potential of these characteristics, and the *rII* region has subsequently become one of the most extensively studied genetic regions in any organism.

Nevertheless, little is known about the role of the *rII* gene products in the phage life cycle. This is partly due to the difficulty of isolating these proteins, which were only recently demonstrated to reside in the cell membrane (WEINTRAUB and FRANKEL 1972; PETERSON, KIEVITT and ENNIS 1972; ENNIS and KIEVITT 1973). In addition, it is now clear that the *rII* proteins have a variety of effects, including interaction with a number of other phage and host products (see DISCUSSION for more detail). Investigation of such interactions is currently one of the principal means for studying the role of the *rII* products.

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This paper reports the properties of two phage mutations which partially suppress the lethal phenotype of *rII* mutations for growth in lambda lysogens. These mutations were obtained during the isolation of viable T4 deletions that affect plaque morphology and were originally referred to as the single mutants in Classes III and IV (HOMYK and WEIL 1974). In the experiments of that study, these mutants appeared to cause an increase in the frequency of terminal redundancy heterozygotes of the *rII* region, suggesting that they were deletion mutations. However, electron microscopy of heteroduplexes of their DNA failed to reveal any evidence of deletions. The subsequent discovery that these mutations partially suppress the lethal *rII* phenotype provided an explanation for the apparent discrepancy; in the test which had been used, partial suppression of the *rII* phenotype produces the same effect as does an increase in the frequency of terminal redundancy heterozygotes.

Subsequent experiments showed that these two mutations are similar, if not identical, to a class of mutants previously described by FREEDMAN and BRENNER (1972). FREEDMAN and BRENNER's mutants were detected by their ability to suppress *rII* mutants to the extent of allowing plaque formation. However, this only occurred under conditions in which the exclusion of *rII* mutants was marginal (i.e., *rII* amber mutants in ochre-suppressing hosts and some *rII* missense mutants in certain hosts in which the *rII* mutation is "leaky"). Because they observed suppression only under marginal conditions, FREEDMAN and BRENNER suggested that some *rII* product is required for suppression to occur. By using a more sensitive test, we show in the present paper that low-level suppression can occur even in the complete absence of *rII* products.

FREEDMAN and BRENNER called their mutants "*si*$^+$". In order to retain their basic designation while conforming to the standard rules of nomenclature (DEMEREC *et al.* 1966), we redesignate these mutations as "*sip*" (wild-type allele *sip*$^+$).

The interaction between *sip* and *rII* mutants appears to be part of a complex system of gene interactions. In the first place, DWIGHT HALL (personal communication) has shown that the *sip* mutants are similar to certain *f*olate *a*nalog *r*esistant (*far*) mutants that have been shown to have a pleiotropic effect on the regulation of a number of T4 early genes (JOHNSON and HALL 1974; CHACE and HALL 1975). Second, we have found that large plaque-producing mutations at two sites, designated L_1 and L_2, interact with the *sip* mutations. They reverse the poor phage growth caused by *sip* and, in the one case tested, reverse the *rII*-suppressing ability of *sip*. Furthermore, the mutation L_2 also appears to have a growth disadvantage in *E. coli* CR63 which is partially overcome by mutations at an unlinked site, M.

MATERIALS AND METHODS

Except as indicated below, materials and methods are those given by HOMYK and WEIL (1974).

Bacterial strains: Strain KB (BENZER and CHAMPE 1961) was obtained from S. CHAMPE.

Bacteriophage: Unless otherwise noted, all strains are in T4D. Amber tA3, gene *t* (JOSSLIN 1970) was obtained from W. WOOD and I. LIELAUSIS. The remaining amber mutants (EPSTEIN

et al. 1963) are from the collection of G. MOSIG. *r*263, *r*HB6 and *r*AP219 (BENZER and CHAMPE 1961) were provided by S. CHAMPE in T4B. *r*263 and *r*1272 (BENZER 1961) were backcrossed into T4D as previously described (HOMYK and WEIL 1974).

One-step growth experiments: Exponential cultures of K(λ) were grown in HB at 37° and concentrated to 1×10^9 cells/ml in fresh HB. The cells were made 4×10^{-3} M NaCN and chilled on ice for 5 minutes. 0.4 ml cells were mixed with 0.4 ml phage to give a multiplicity of infection of 0.02 or 0.5 and adsorbed for 20 minutes at 37° with gentle shaking. Unadsorbed phage were removed by antiserum, 5 minutes, K=3. The cells were washed with 10 volumes cold HB, resuspended in 10 volumes cold HB and several dilutions were plated on B to obtain the titre of total plaque-forming units. A sample was also diluted 10-fold in warm HB and aerated for 120 minutes at 37°. Samples taken at 30-minute intervals were treated with chloroform and plated to obtain the total number of plaque-forming units, and hence a crude growth curve. Assays were also made for phage adsorption, unadsorbed phage remaining after antiserum treatment and cell washing, and cell recovery after washing. Corrections were made for these factors, as well as for the calculated frequency of multiple infection in those experiments with nominal m.o.i. = 0.5.

RESULTS

rII *suppression by* sip1 *and* sip2. The ability of *sip1* and *sip2* to suppress the lethal phenotype of *rII* deletion mutants was tested by examining a single round of infection in a restrictive host. Phage were adsorbed to *E. coli* K(λ) and the proportion of productively infected cells and average burst size was determined. Results are given in Table 1 for *sip* suppression of *r1272*. It will be seen that the presence of either *sip* mutation increases the frequency of productive infections by 20 to 40-fold over the *sip*$^+$ control. However, the absolute level of suppression is low: between 5 and 15% of the infected cells produce a burst of particles, and the average yield of particles per cell is about one. This low level of suppression is consistent with the fact that the *r1272 sip* double mutants do not form plaques on K(λ).

The *r1272* mutation deletes the entire *rII* region (BENZER 1961) plus the adjacent D_1 region including the *den* B gene (VETTER and SADOWSKI 1974). In addition, similar levels of suppression were obtained with *r1236*, which has a deletion in the B cistron (see WEIL and TERZAGHI 1970), and with *r1589*, which makes a hybrid gene product having only B-cistron activity (CHAMPE and BEN-

TABLE 1

*Phage production on K(λ)**

Phage strain†	Experiment 1 Transmission coefficient‡ (%)	Experiment 1 Burst size§	Experiment 2 Transmission coefficient‡ (%)	Experiment 2 Burst size§
r1272	0.4	31.5	0.2	4.5
r1272 sip1	11.0	1.3	4.6	0.7
r1272 sip2	15.0	0.7	8.4	0.4

* See MATERIALS AND METHODS for experimental details.
† Phage strains in T4D.
‡ Fraction of adsorbed phage which gave rise to a plaque on the permissive host. (Adsorption to K(λ) was greater than 95%).
§ Yield of phage particles divided by fertile cells as determined from the Transmission coefficient.

ZER 1962). These results indicate that the level of suppression observed here does not depend on the presence of functional *rII* gene products.

Effect of sip1 *and* sip2 *on plaque morphology.* Because of the manner in which they were isolated (HOMYK and WEIL 1974), *sip1* and *sip2* were first obtained in T4B as double mutants both with *r1589* and with *r1236*. On *E. coli* B, *r1589 sip1* and *r1236 sip1* make clear plaques that are somewhat smaller than those of *r1589* or *r1236* and rather variable in size. *r1589 sip2* and *r1236 sip2* produce small turbid plaques, also variable in size.

Two lines of evidence indicate that the same mutations produce altered plaque morphology and *rII* suppression. First, the plaque morphology markers of *r1589 sip1* and *r1589 sip2* were transferred into a T4D *r1589* genetic background in two backcrosses in which the *sip* parent was heavily irradiated with ultraviolet light (see HOMYK and WEIL 1974). The resulting T4D strains had the same transmission coefficient on K(λ) as did the original T4B strains (data not shown). Second, the *r1272 sip* strains mentioned above were constructed in a cross of *r1589 sip* × *r1272*. *Sip* was selected on the basis of plaque morphology, but the resulting strains showed *rII* suppression (Table 1).

The *sip* mutations were separated from *rII* by crossing the two *r1589 sip* strains to T4D wild type. In each cross, recombinants which produce typical *rII* plaques occurred at a frequency of about 12%. The reciprocal recombinants were found at approximately equal frequency. They produce turbid plaques that are variable in size and less than 1/4 the diameter of T4D wild type on *E. coli* B.

In the genetic crosses described below, *sip* genotype was determined on the basis of plaque morphology.

Genetic mapping of sip1 *and* sip2. Before we became aware of the paper of FREEDMAN and BRENNER (1972) we performed a series of genetic crosses to determine the map location of the *sip* mutations. Preliminary mapping experiments placed the mutants in the vicinity of gene *52* (data not shown). Following these preliminary experiments, a set of crosses was performed to determine the precise location of *sip1*. The data are presented in Figure 1. The data clearly establish that the *sip* mutations lie to the right (clockwise on the standard map) from gene *t*, but there is ambiguity with respect to the relative locations of *sip* and *amH17* (gene *52*).

Quantitative determination of recombination between *sip1* and *sip2* is made difficult by the fact that stocks of these strains always contain a significant proportion (0.1 to 4%) of phage which produce large plaques (see below). The following evidence indicates, however, that *sip1* and *sip2* are at different, but closely linked, sites. Several stocks of each of the phage strains *sip1*, *sip2*, *r1589 sip1* and *r1589 sip2* were prepared. For each strain, the stock having the lowest frequency of large plaque "pseudorevertants" was chosen and crosses were performed to test for recombination between *sip1* and *sip2*. The results of these crosses and of controls are presented in Table 2. As can be seen, the frequency of large plaque-forming phage in each of the stocks and in the control crosses was much less than 1%, while the fraction of large plaque producers in the progeny of crosses *sip1* × *sip2* and *r1589 sip1* × *r1589 sip2* was 1.0 and 1.4% respectively.

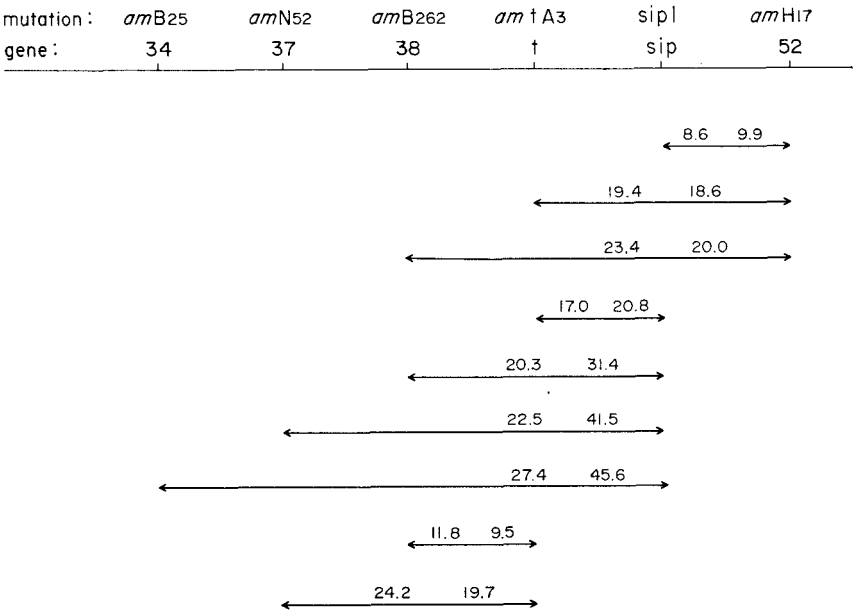

FIGURE 1.—Mapping of *sip1*. Crosses between amber mutants were plated on B to determine *am*+ recombinants and on CR63 for total progeny. Crosses involving *sip1* were plated on B and large plaques were scored as *am*+ *sip*+; total progeny were determined on CR63. Values given are for duplicate crosses: 200 × scored recombinants/total progeny. Values on the left side are all from one experiment; values on the right are all from a second.

It thus appears that there is approximately 2% recombination between the two *sip* mutations.

The data presented thus far indicated three similarities between the *sip* mutants and the mutants described by FREEDMAN and BRENNER: production of small plaques, low-level suppression of *rII*-lethality (measured in different ways in the two cases), and general map location. It was therefore important to compare the two types of mutants more closely.

TABLE 2

Recombination between sip1 *and* sip2*

Cross or stock lysate	No. of large plaques	Total no. of plaques	Percent large plaques
rII+ *sip1* stock	14	4952	0.28
r1589 sip1 stock	0	1459	<0.07
rII+ *sip2* stock	3	3312	0.09
r1589 sip2 stock	0	1000	<0.10
rII+ *sip1* × *rII*+ *sip1*	3	1500	0.20
rII+ *sip1* × *r1589 sip1*	1	823	0.12
rII+ *sip2* × *r1589 sip2*	2	2000	0.10
rII+ *sip1* × *rII*+ *sip2*	75	7423	1.0
r1589 sip1 × *r1589 sip2*	41	2905	1.4

* Crosses were performed in B and the progeny were analyzed by plating on B.

We isolated three independent mutations using the methods and strains reported by FREEDMAN and BRENNER (1972): Three *rII* missense mutants in T4B, *r263*, *rHB6* and *rAP129* were plated on *E. coli* KB, on which they are poorly restricted. One small-plaque pseudorevertant was picked from each phage strain, and in each case it was verified that the resulting isolate carried both the original *rII* mutation and a second mutation conferring small plaque size even in the absence of *rII*.

Since *sip1* and *sip2* as well as the new isolates produced small plaques, plaque phenotype was used as the criterion for investigating recombination. Among the progeny of all pairwise crosses of the T4B *r263*, *rHB6* and *rAP129* pseudorevertant isolates to one another and to T4B *r1589 sip1* and *r1589 sip2*, fewer than 1% large plaque-producing phage were observed. Thus *sip1* and *sip2* map close to mutations isolated by the method of FREEDMAN and BRENNER. More accurate mapping was precluded by the fact that all the strains involved contained a significant proportion of phage which produce large plaques.

To determine whether the *sip* mutations can suppress *rII* to the extent of allowing plaque formation under conditions of marginal *rII* restriction, the double mutants T4D *r263 sip1* and T4D *r263 sip2* were constructed. These double mutants formed very small plaques on KB with an efficiency of plating (compared to titre on *E. coli* B) of 0.4 and 0.3 respectively. In a control plating of T4D *r263* on KB, the efficiency of plating was less than 5×10^{-4}. Thus *sip1* and *sip2* can suppress to the level of plaque formation under the same conditions as do the mutants isolated by FREEDMAN and BRENNER.

On the basis of their similar phenotypes and map location, we conclude that *sip1* and *sip2* are identical to the mutants reported by FREEDMAN and BRENNER. The combined mapping data of the two investigations places the mutants between genes *t* and *52*.

Pseudorevertants of sip1 *and* sip2. As previously discussed, stocks of *sip* and *rII sip* contain a rather high frequency (0.1 to 4%) of particles which produce large plaques. Presumably these are due to mutations that confer a selective growth advantage under the liquid growth conditions used to make stocks.

Two large plaque-formers were isolated by plating *r1272 sip2* and *r1589 sip2* on *E. coli* CR63. The resulting strains were designated *r1272 sip2* L_1 and *r1589 sip2* L_2 respectively. Both these strains produce large clear plaques on CR63, suggesting an absence of lysis inhibition. This is in contrast to the parental strains and to *rII* mutants, which produce smaller turbid plaques. The plaque morphology of *r1272 sip2* L_1 and *r1589 sip2* L_2 on B is identical to that on CR63.

The single mutants L_1 and L_2 were isolated from crosses to T4 wild type. They produce identical, large "rapid lysis" ("r") plaques on *E. coli* B. The plaques have slightly turbid halos and are distinguishable from *rI*, *rII*, and *rIII* plaques. The "r" plaque phenotype of both L_1 and L_2 is enhanced by prolonged incubation at 37° or by incubation at 43°.

Crosses were performed between L_1 and L_2, L_1 and *sip2* and L_2 and *sip2*. Linkage was not detected in any case. From the first cross we obtained the presumed L_1L_2 double mutant at approximately the same frequency as wild-type recombinants. The double mutant produces plaques with large turbid halos.

TABLE 3

Effect of L_1 on sip2 *suppression**

Phage strain	Transmission coefficient†	Transmission coefficient†
r1272	0.02	0.1
r1272 sip2	4.6	4.6
r1272 sip2 L_1	0.7	0.1

* See MATERIALS AND METHODS for experimental details.
† Percent of adsorbed phage which gave rise to a plaque on the permissive host. Results of two independent experiments. Adsorption to K(λ) was greater than 95%.

We examined the effect of L_1 on the ability of *sip2* to suppress *r1272* for a single cycle of growth in K(λ). As can be seen in Table 3, the triple mutant *r1272 sip2* L_1 has the same low transmission coefficient as does *r1272*. Thus L_1 eliminates suppression by *sip2*.

A mutation that interacts with L_2. Several independent stocks of L_2 were found to contain a significant proportion (0.1 to 1.0%) of phage carrying an additional mutation. On *E. coli* B these phage produce plaques which are about one half the diameter of L_2 plaques and are much clearer. A strain designated L_2 M_1 was obtained from one such plaque.

When a cross between L_2 M_1 and T4 wild type was plated on B, recombinant L_2-type plaques were found with a frequency of about 14%, indicating little or no linkage. The only other plaques were parental types. We have not carried the analysis further, but it appears that M_1 alone is either lethal or else produces plaques that are indistinguishable from L_2M_1, L_2 or wild type. The reoccurrence of such mutants in L_2 stocks indicates that they confer a selective growth advantage on L_2.

DISCUSSION

We have isolated two mutants, designated *sip*, which partially suppress the lethality of T4 *rII* mutations for growth in lambda lysogens. When tested for the ability to allow a single cycle of growth under highly restrictive conditions (i.e., with *rII* deletions), the *sip* mutants increase by twenty- to forty-fold the fraction of infected cells that produce phage. However, the proportion of productive cells is still low (ca. 15%), and the burst size from such cells is about one. This level of suppression is insufficient to allow plaque formation. When tested with the leaky mutant *r263*, the *sip* mutants do allow plaque formation on a number of hosts on which *r263* alone cannot form plaques. Both *sip* mutants have a lengthened eclipse period and an increase of 10–12 minutes in the latent period (unpublished data), but the two mutants have slightly different effects on growth and plaque type. *rII sip1* phage produce smaller than normal, but "rapid lysis" or "r" type plaques; *rII sip2* mutants produce even smaller, r^+ type plaques. The burst size of *rII sip1* phage also averages 20–30% higher than that of *rII sip2* (unpublished data). There is approximately 2% recombination between the two mutants, and they map about one-third of the distance from gene *52* to gene *t*.

Sip1 and *sip2* are clearly similar, if not identical, to the low-level *rII* suppressors isolated by FREEDMAN and BRENNER (1972). Those suppressors were isolated by virtue of their ability to allow plaque formation by *rII* mutants such as *r263* on *E. coli* KB and certain other hosts. *Sip1* and *sip2* have the same property. In addition, suppressor mutants that we isolated by the method of FREEDMAN and BRENNER were shown to give no more than 2% recombination with *sip1*.

The *sip* mutants also appear to be similar to a recently described class of *f*olate *a*nalog *r*esistant (*far*) mutants (JOHNSON and HALL 1974). D. HALL (personal communication) has shown that *sip1* and *farP85* show less than 0.5% recombination, and that *sip1* is folate analogue resistant. *FarP85* and a closely linked mutant *farP14* have pleiotropic effects on the regulation of a number of T4 genes (JOHNSON and HALL 1974; CHACE and HALL 1975). By virtue of this characteristic, plus similarity of map position, they also appear very similar to the temperature-sensitive mutant *tsG1* reported by MATTSON, RICHARDSON and GOODIN (1974). Characteristic of *tsG1* and the *far* mutations is an enhanced production and/or increased period of synthesis of immediate early gene products and a reduction and/or delay in the synthesis of delayed early gene products. It has been hypothesized that these mutations reduce the efficiency with which delayed early promoters are utilized in RNA transcription (HERCULES and SAUERBIER 1974; MATTSON, RICHARDSON and GOODIN 1974; CHACE and HALL 1975).

FREEDMAN and BRENNER (1972), using a plaque assay, were only able to detect *sip* suppression of leaky *rII* mutants. They thus inferred that suppression by *sip* depends upon the presence of a low level of functional *rII* products. Since the *far* and *tsG1* mutants cause overproduction of *rIIA* protein, and *farP14* also overproduces *rIIB* protein (MATTSON, RICHARDSON and GOODIN 1974; CHACE and HALL 1975), FREEDMAN and BRENNER's hypothesis is entirely plausible. However, by using a more sensitive test we have shown that *sip* can also suppress *rII* deletions, including the mutant *r1272*, which eliminates the entire *rII* region (as well as the adjacent D1 region and gene *denB* (endonuclease IV) (VETTER and SADOWSKI 1974)). Thus *sip* causes some alteration in the infectious cycle which partially overcomes the *rII* lethal block even in the complete absence of *rII* products. Clearly, this effect may also contribute to the ability of leaky *rII* mutants to grow sufficiently well to form plaques.

Several lines of evidence indicate that the *rII* products cause an alteration in the bacterial cell membrane (GAREN 1961; FERRO-LUZZI-AMES and AMES 1965; SÉCHAUD, KELLENBERGER and STREISINGER 1967; BULLER and ASTRACHAN 1968; KRYLOV 1970) and *rII* products have recently been shown to be present in the membrane (WEINTRAUB and FRANKEL 1972; PETERSON, KIEVITT and ENNIS 1972; ENNIS and KIEVITT 1973; HUANG 1975). In addition, *rII* mutations interact with mutations in a number of other T4 genes: gene *30* (BERGER and KOZINSKI 1969; EBISUZAKI and CAMPBELL 1969; KRISCH, SHAH and BERGER 1971; KARAM and BARKER 1971); *su30* (KRYLOV 1972), gene *32* (MOSIG and BRESCHKIN 1973, 1975), *t* (JOSSLIN 1971; KRYLOV 1971) and *stIII* (KRYLOV and YANKOFSKY 1975). Many of these interactions also suggest that *rII* products function at the membrane.

The work reported in this paper demonstrates additional interactions between *rII* and other phage gene products. The two mutations designated L_1 and L_2 reverse the growth defect of *sip* and, by themselves, produce rapid lysis plaques. Furthermore, in combination with *rII* and *sip* mutations, the triple mutants *rII sip1* L_1 and *rII sip2* L_2 produce "r" type plaques on *E. coli* CR63, on which *rII* and *rII sip* mutants produce turbid plaques. In the only case tested, it was shown that L_1 also reverses the ability of *sip2* to suppress the *rII* lethal phenotype. A further mutant, designated M_1, improves the growth of T4 L_2 on CR63, and has an effect on plaque morphology.

The apparent identity of the *sip* mutants and the *far* and *tsG1* mutants indicates that the *sip* mutations may affect the synthesis of many gene products. Thus there are many possible mechanisms by which they might provide partial suppression of the *rII* lethal phenotype. The ability of L_1 and L_2 to affect the *sip* mutations is of interest. Their rapid lysis plaque morphology suggests that they affect lysis inhibition, which in turn suggests an effect on the cell membrane. There are two general mechanisms by which they might alter the phenotypic effects of the *sip* mutations. On the one hand, they may reverse the effect that *sip* mutants have on gene regulation, returning gene expression to a pattern more similar to that which occurs in sip^+ infections. On the other hand, they may have no effect on gene regulation, but instead may interact with the products of one or a few of the *sip*-affected genes, thus altering phage growth and *rII* suppression. In either case, further investigation should prove useful.

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