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**Bacteriophage P2: recombination in the superinfection  
preprophage state and under replication control by phage P4**

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**Abstract** Genetic crosses (mixed infection, lytic cycle) with bacteriophage P2 are known to give extremely low recombination frequencies, and these are unaffected by the *recA* status of the host bacterium. We now show the following: (a) satellite bacteriophage P4, which interacts with P2 in a number of ways, but is quite different from it in terms of DNA replication and controls, is clearly dependent on the host *recA*<sup>+</sup> function for recombination; (b) a chimeric phage (Lindqvist's P2/P4 Hy19), where P2 replication early genes have been replaced by those of P4, does recombine in a *recA*<sup>+</sup> dependent manner; (c) immunity sensitive P2 phages, mixedly infecting P2-immune bacteria, hence blocked in their replication, do recombine in a *recA*<sup>+</sup> dependent manner; (d) an analysis of exchange distribution based on a simple model confirms that in mixed infection of sensitive cells (where P2 is actively multiplying) recombinational exchanges tend to be statistically clustered in a segment of the chromosome containing the origin of replication, and also shows that under conditions of blocked P2 DNA replication the distribution of exchanges correlates well with the physical distances between markers on the P2 DNA.

**Key words** Mapping · Crosses · Replication origin · *recA* · Midwife phage

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## Introduction

The capacity to recombine is one of the most general properties of genetic material and has obvious evolutionary implications for the generation of new genotypes. Recent advances have revealed a bewildering array of molecular mechanisms implicated in recombination. The successes of classical genetics now seem miraculous when one considers that for more than half a century its main analytical tool was the measurement of recombinant frequencies in crosses, a tool based on the assumed correlation between frequency of recombination and chromosome distance. Many of the same recombination mechanisms are also involved in repair of damaged DNA and recent work (reviewed by Cox et al. (2000), Mosig (1998), Kuzminov (1999), and in a dedicated issue (NN, 2000) of *Trends in Biochemical Sciences*) convincingly shows that they play an essential role in DNA replication.

At the molecular level, recombination phenomena may be either site-specific (occurring only at unique DNA sequences), or unspecific (or only mildly specific, as for *chi* sites, see Stahl, 1979) as to sequence. The latter category more closely corresponds to the classical concept of genetic recombination, traditionally assumed to be a random event along the length of a chromosome. As first shown in *Escherichia coli* (Clark and Margulies, 1965) and later found in several other organisms, such recombination is strongly dependent on the presence of a normal *recA* (or *recA* like) gene. This general mechanism of recombination usually operates also upon guest genetic elements, like plasmids or bacteriophages, that may be present in the cell. Several phages and plasmids however encode some recombination functions and these may interfere with those of the host cell.

Bacteriophage P2 appears to be exceptional in this respect. In ordinary phage crosses (*i.e.* mixed infection of sensitive cells, lytic cycle), it shows extremely low frequencies of genetic recombination (Lindhahl, 1969a,b) (site specific recombination is not considered here), and these are invariant with the *recA* (Hudnik-Plevnik and Bertani, 1980) or *recB* (Lindhahl, 1969b) status of the host bacterium. [More precisely, Lindahl used the *hyd* mutant of Sironi (1969), later shown (Sironi et al. 1971) to be allelic to *recB*.] The P2 related phage 186 seems to behave like P2 in this respect (Hocking and Egan, 1982), and so do to some extent the minute bacteriophages S13 and  $\Phi$ X174 (reviewed by Warner and Tessman, 1978). Perhaps

unique characteristics of the mechanism of replication of P2 DNA make it refractory to recombination. If this view were correct, one might expect that P2 DNA, either replicating under the control of another replicon, or not actively replicating, would undergo normal recombination. These predictions are tested in this work. The alternative replicon used here, bacteriophage P4, although functionally interacting with P2 as a satellite virus (Six and Klug, 1973), is quite different from P2 in its DNA replication. The extensive information available on bacteriophages P2 and P4 has been reviewed by L.E. Bertani and Six (1988), Christie and Calendar (1990), and Lindqvist et al. (1993), and we refer to them for our otherwise unsupported statements. For our purpose, it was important to also establish that P4, unlike P2, is unexceptional in respect to recombination.

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## Materials and methods

Properties and derivation of bacteria, bacteriophages and plasmids used in this work, are given in Table 1.

Crosses and other manipulations with phage P4 were done according to Dehò (1983), except for the following: incubation was at 37°C, 20 min were allowed for adsorption, followed by a 10<sup>4</sup>× dilution and 150 min incubation, at the end of which the lysate was treated with chloroform. Burst sizes were between 40 and 60. In the isolation of the parent phages for cross 2 of Table 2 (see Table 1), enrichment for *del24* types was obtained by heat exposure (Dehò, 1983).

Crosses and other manipulations with phage P2 (and P2/P4 Hy) were done essentially according to Bertani (1975) and Hudnik-Plevnik and Bertani (1980). Incubation was generally at 30°C, except that in crosses of P2/P4 Hy derivatives and some controls (Table 2, lines 4 through 9) after 15 minutes adsorption at 30°C the temperature was shifted to 37°C. In these crosses the burst size varied between 10 and 60, subnormal for P2 (perhaps because the crosses were done under complementation conditions for the *tsD4* marker) and, unlike the P4 case, was apparently indifferent to the *recA* status of the host. For the isolation of new recombinant types (crosses mentioned in Table 1), the frequency of recombination was increased by means of ultraviolet light irradiation (UV crosses). This was done either by exposing the parent phages to 400

ergs/mm<sup>2</sup>, 254 nm UV (see Fig. 2 of Lindahl, 1969b), or by irradiating the mixedly infected bacteria, after adsorption and resuspension in UV transparent buffer and before dilution in broth and incubation.

In several crosses the frequencies of the selected recombinants were quite low. Care was taken in such selective assays to dilute as much as practically possible (and use large numbers of plates) in order to exclude spurious effects from mixed infection on the plates. Also, relatively high concentrations (up to  $2 \times 10^8$ /ml in the soft agar layer) of indicator bacteria were used for such selective assays.

Distances between markers, and total chromosome lengths, are based on DNA sequence data (see Fig. 1), and take into account deletions, if any are present.

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## Results<sup>(1)</sup>

The linear, double stranded DNAs of P2 and P4 phage particles circularize through their cohesive ends upon injection and replicate as circular, usually monomeric molecules. Apparently, only at encapsidation are the DNA circles specifically cut to form again the free cohesive ends which are identical for the two phages (Pruss et al. 1975). One should then expect that the recombining elements be usually circular DNA molecules, which imposes additional limitations, as compared to linear chromosomes, on what one may quantitatively infer from recombination frequencies (see Stahl, 1967). The genetic maps of both bacteriophages are circular (Bertani, 1975; Dehò, 1983). Both bacteriophages are able to establish lysogeny by inserting their circularized DNA into the bacterial chromosome *via* site specific recombination. The latter requires proteins specified by the respective *int* phage genes, and may also operate on two homologous phage chromosomes following mixed infection. To examine generalized (as opposed to site specific) recombination in these phages, it is advantageous to use phage mutants whose *int* gene is either absent or inactive, for example deletion mutants *vir22*, *del6* or *vir79* in P2 and *del24* in P4.

In crosses of phage P2 *vir22* in the host *E. coli* C-1757, the frequency of wild type recombinants between P2 genes D and L (which are nearly opposite to each other on the circular map; see Fig. 1), was found to be  $5.6 \times 10^{-4}$  (S.E. =  $0.6 \times 10^{-4}$ ; N=16) (E. Capaccio, unpublished experiments done under the same conditions as used here for P2 crosses, at

30°C). The same cross, with the *E. coli* K-12 derivative K-221 as the host, gave  $3.6 \times 10^{-4}$  (S.E. =  $0.4 \times 10^{-4}$ ; N=4) recombinants (Hudnik-Plevnik and Bertani, 1980, average of crosses 1b, 2, 3, and 4a in their Table 2). The two host strains used, C-1757 and K-221, differ in numerous properties, but are both *recA*<sup>+</sup>. Using a *recA*<sup>-</sup> derivative of K-221 (*ibidem*, cross 7a) or a *recA*<sup>-</sup> hybrid between C and K strains (*ibidem*, cross 8a) as the host, did not seem to make any difference in the recombinant frequency. The presence of the *del2* deletion (crosses of line 3, Table 2) did not significantly affect the recombinant frequency between L and D, nor did performing the cross at 42°C rather than 30°C (data not shown).

In contrast to the P2 case, the effect of *recA*<sup>-</sup> is quite strong in P4 *del2*<sub>4</sub> crosses. Using a derivative of K-221 as the host, the frequency of wild type recombinants between P4 genes  $\alpha$  and  $\epsilon$  (see Fig. 1) was 100× higher in the *recA*<sup>+</sup> host than in *recA*<sup>-</sup> (Table 2, cross 2). The effect was still evident in cross 1, Table 2, where the absence of the deletion allowed site specific recombination presumably also to occur. Thus P4 behaves like most other phages studied and is unlike P2 in this respect.

Notwithstanding their similarities with respect to circularization and encapsidation, DNA replication is quite different in these two phages. It is unidirectional in P2 (Schnös and Inman, 1971), bidirectional in P4 (Krevolin et al., 1985); in P2 it requires host genes *rep* (Calendar et al. 1970), *dnaB* and *dnaG* (Bowden et al. 1975), not so in P4 (Lindqvist and Six, 1971), not to speak of the unrelated phage genes involved in the process. Lindqvist (1981a, b) succeeded in constructing a hybrid, *alias* chimeric phage, P2/P4 Hy19, where the P4 sequences essential for DNA replication are present together with all P2 sequences required for capsid formation, while all P2 genes known to be needed for P2 DNA replication are absent. P2/P4 Hy19 is thus no longer a satellite phage, since it can multiply independently of the presence of other P2 genes in the host cell. Like P4, it does not require the *rep* host gene (Lindqvist, 1981b). We asked whether recombination in this hybrid phage would be of the P2 or of the P4 type. By appropriate crosses (Table 1) markers in genes *D* and *L* were introduced into Lindqvist's chimeric phage, and these constructs were then used to test for recombination in the presence or absence of the *recA* function in the host bacteria, as done with P2 and P4. The data (Table 2, crosses 4 through 9) clearly show that recombination in the P2 segment of the chimeric phage is strongly dependent on the host bacterium being *recA*<sup>+</sup>

and is then much higher, for the same markers, than in the original P2 parent. [Unexpectedly, a minor reduction in the frequency of recombination with P2/P4 Hy19 was noted (Table 2, crosses 5, 7, and 9) when the host was defective in the *rep* gene.]

If the recombination refractoriness of P2 were a consequence of its DNA replication mechanism, one might expect that non-replicating P2 DNA would recombine normally in presence of the recombination functions of the host cell. In fact, observations incidental to *E. coli* C mapping (Wiman et al. 1970) had suggested a normal rate of recombination within P2 prophages (i.e. inserted in the bacterial chromosome) in conjugational crosses between two appropriately marked lysogenic bacteria. We tested that expectation more stringently by measuring recombination between non-replicating P2 genomes, taking advantage of the fact that the P2 genome superinfecting a P2-immune cell is essentially blocked in its replication by the action of the P2 immunity repressor. As "superinfection preprophage" (Bertani, 1953, 1954), it survives largely inactive during several rounds of host cell duplication and is distributed among the daughter cells. If one of these cells shifts to lysis, the superinfection preprophage, or at least some of its genes, may be represented among the phage progeny (*ibidem*). In order to minimize possible genetic interactions between superinfecting phage DNA and prophage DNA, instead of lysogenic cells we used cells carrying a multicopy plasmid which expressed an inserted P2 repressor gene (Westöö and Ljungquist, 1980). The presence of the plasmid does not seem to affect the recombination frequency in ordinary crosses (Table 2, line 10). The plasmid-bearing bacteria were mixedly infected (Infection I) at relatively high multiplicities with P2 phages defective in either gene L or D. To avoid complications due to site specific recombination both phages carried deletion *del6* (L E Bertani, 1980) which removes part of gene *int* and damages the phage attachment site. The immunity insensitive P2 derivative P2 *del6 vir79*, also made defective in both genes D and L (Table 1), was used as the midwife phage to infect (Infection II) and lyse cultures carrying the preprophages. At this time most of the cells would be either free of any preprophage or carry just one copy, and the experiment would measure how many of the preprophages that happen to be encapsidated thanks to the midwife phage had undergone recombination. A more detailed description of this rather elaborate experiment is given under Table 3.

The experiments answered quite clearly the question posed. Recombination did take place between replication-blocked, non-integrated phage genomes in *recA*<sup>+</sup> host bacteria, while it did not (or did with an at least 100× lower efficiency) in the *recA*<sup>-</sup> host (Table 3, last three rows). The following comments concern several other aspects of the experiments of Table 3.

(a) Immunity to superinfection of lysogenic bacteria is not absolute and may break down with productive lysis in some superinfected cells, in a manner dependent on the phage genotypes involved and on the multiplicity of superinfection (Bertani, 1954; L E Bertani, 1961). In the present experiments this may also have occurred as a result of fluctuations in the plasmid copy number. Indeed, the amount of free phage present in the cultures being prepared for Infection II was higher than could be explained as due to residual unadsorbed phage from Infection I. Furthermore, it included a small proportion (between  $6 \times 10^{-5}$  and  $1.2 \times 10^{-3}$ ) of *am*<sup>+</sup> *ts*<sup>+</sup> recombinants. Assuming a burst size between 50 and 100, the number of superinfected bacteria lysing productively during the incubation period prior to Infection II can be calculated to be between 1% and 4% of the original Infection I bacterial input. In all experiments of Table 3, with the exception of experiment 11, this free phage was removed by centrifugation in preparing the cultures for Infection II. Thus in all such cases, the amount of phage carried over with the pellet was too small to affect significantly the frequencies of phage types resulting from Infection II.

(b) In Table 3 the number of expected preprophages at Infection II was calculated on the assumption that they underwent no replication and no decay. In older experiments (Bertani, 1954) with P2 (in *Shigella*, rather than *E. coli*, as the host), superinfected *lysogenic* bacteria gave a preprophage recovery approaching 100% when *spontaneously* (that is, in the absence of midwife phage) shifting to the lytic cycle. Such a shift would require the spontaneous loss of immunity (mechanism still unknown) and allow multiplication of the preprophage prior to lysis, whereas in the present context the midwife phage is insensitive to the immunity repressor, while the preprophage would not be expected to multiply before being encapsidated. The recovery of parental types rescued by the midwife phage was measured with some precision only in experiment 11 of Table 3 by eliminating the midwife phage in separate selective platings for *am*<sup>+</sup> or *ts*<sup>+</sup> types. The two parental types were present

in roughly equal amounts, as expected, and together represented at most 40% of the preprophages expected. In the other experiments of Table 3 this measurement was not made, but calculations based on the presence of a few turbid plaques among those (typically small, sharp and clear) of the midwife phage suggest that the rescue frequency was even lower, of the order of a few percent of the preprophages. The source of this difference was not investigated.

(c) The frequency of recombinant phage as given in the penultimate row of Table 3 cannot be directly compared with the frequencies of recombinants observable in standard crosses, as exemplified in Table 2. In the latter case, a number of actively replicating phage DNA copies are exposed to reciprocal interactions for a limited time, shorter than the latency period. In the superinfection experiments of Table 3, the repressed copies of phage DNA could in theory interact during the whole duration of the experiment; however, their number per cell is expected to rapidly decrease to either one or zero as the cells divide, after which interactions would be impossible.

(d) The burst sizes for the *vir79* phage in the experiments of Table 3 and in the crosses of line 10 in Table 2, where the same genetic markers were present, were significantly reduced in the *recA*<sup>-</sup> host. On the other hand, in crosses 1 through 6 of Table 2 there was no significant reduction of burst size in the *recA*<sup>-</sup> host. We do not know the reason for this difference; perhaps it reflects some peculiarity of the *vir79* mutation.

(e) A simple model of recombination between two homologous DNA rings will assume the occurrence of two reciprocal exchanges to reestablish monomeric rings, which are the main replication intermediates (Lindqvist, 1971; Schnös and Inman, 1971; Kainuma-Kuroda and Okazaki, 1975; Kurosawa and Okazaki, 1975) and the preferred encapsidation substrate (Pruss et al. 1975) in P2. It was possible to study the distribution of one of these exchanges since the two parent phages, used in the experiments of Table 3 and in the corresponding standard crosses 11 and 12 of Table 2, differ in a third, unselected marker, *tsC5* as opposed to *C*<sup>+</sup>. These two allelic types are distinguishable in the *am*<sup>+</sup> *tsD*<sup>+</sup> selective platings at 42°C to 43°C. The frequencies of turbid (*C*<sup>+</sup>) plaques among the *am*<sup>+</sup> *tsD*<sup>+</sup> recombinants recovered (Table 4, Part I) were apparently independent of whether the plasmid carried by the host was of the *C*<sup>+</sup> or the *tsC5* type. This suggests that genetic exchanges between the

superinfection prephages and the short homologous segment on the plasmids were few if any, and may be neglected. One can then compare those frequencies with theoretical expectations based on the map positions of the three markers involved, *amL302*, *tsD4*, and *tsC5*. With reference to the map positions of the markers (see Fig. 1), one can readily see that a heteroallelic (circular) dimer formed by a reciprocal exchange between *amL302* and *tsD4* will produce an *am<sup>+</sup> tsD<sup>+</sup>* monomer through a second exchange in either the short arc *tsD4* to *tsC5* (yielding a turbid plaque) or in the longer arc (across the origin of replication *ori* and the *cos* joint) *tsC5* to *amL302* (yielding a clear plaque at 42°C). (Although reciprocal prephage crosses were not done, past experience with the *tsC5* mutation would exclude any significant marker effect.) In a simple model these two alternatives for the second exchange ought to occur at relative frequencies proportional to the lengths of the two arcs, which may be calculated from the available DNA sequence and map data (Fig. 1). The variability in the experimental data (Table 4, Part I, B) is high. Moreover, only a range (Table 4, Part I, A) of expected values, rather than a unique theoretical value, may be calculated because the exact nucleotide sites for mutations *amL302* and *tsD4* have not yet been established. The comparison however does not disprove the model. In particular, it gives no indication of an excess of exchanges in the arc that includes the replication origin *ori*. This is of special interest, because standard (mixed infection of sensitive cells, lytic cycle, no UV irradiation) P2 phage crosses had indicated (Bertani, 1975) an excess of recombination in this segment of the P2 chromosome. Those older results are restated here (Table 4, Part 2) and compared with the theoretical expectation that may now be precisely calculated from physical map data. Furthermore, standard crosses (Table 2, crosses 10 and 11), using the same markers as the superinfection experiments of Table 3, demonstrate (Table 4, Part 1, C) even more convincingly the excess recombination in the chromosome segment that includes the replication origin. There is thus a difference in the distribution of exchanges along the P2 chromosome depending on whether it is actively multiplying or is blocked by immunity to superinfection.

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## Discussion

A bacteriophage capacity to recombine in the course of its multiplication is the resultant of a variety of events at the molecular level. Historically, these were first inferred from purely genetic experiments (mutant isolation, survival to radiation, mixed infection, etc.) and recombination mapping was for several decades the main tool for dissecting the structure of genomes. The modern powerful techniques for the isolation and characterization of nucleic acids and proteins have largely superseded those less direct methods. As a consequence, the number of viruses for which recombination data and genetic maps are available is rather small when compared to the host of physical maps obtained through the direct studies.

Among the relatively few phages for which extensive recombination data are available (most T-phages,  $\lambda$ , some *B. subtilis* phages, some of the minute phages, etc.) phage P2 is at the low end of the range for recombination frequency in standard crosses. Furthermore, that frequency is unaffected by the presence or absence of the *recA* and *recBC* functions. The comparison with phage  $\lambda$  (whose DNA is only 1.4 $\times$  larger than P2's) is particularly striking. For distant markers, the recombination frequency in  $\lambda$  may reach 8 to 12% in a wild type host; with  $\lambda$  *red int* it would be reduced to 1 to 3%, but in a *recA*<sup>-</sup> host it would be further reduced a hundred fold.

Attempts to demonstrate specific interactions between multiplying P2 and the recombination mechanisms of the host, that might explain the unusual recombination properties of P2, were inconclusive<sup>(2)</sup>. Besides the very few open reading frames having still unknown functions in P2, genes *old* and *tin* might be suspected of affecting general recombination. Gene *old* is lethal for *recBC* mutant host cells (Sironi et al. 1971; also Ghisotti et al. 1983, and references cited therein), strongly interferes with the multiplication of phage  $\lambda$  (Lindahl et al. 1970), and specifies a nuclease (Myung and Calendar, 1995). Yet, the effect on recombination frequencies, when both parents are *old* mutants, is insignificant. Gene *tin* interferes with the major single stranded DNA binding protein of phage T4 (Mosig et al. 1997). Its effect on recombination has not been tested. On the other hand, seemingly normal recombination levels and *recA* dependence could be demonstrated in several situations deviating from the standard lytic

multiplication cycle of phage P2: prophage to prophage recombination (Wiman et al. 1970), prophage to superinfecting phage recombination (Eastburn, 1969), recombination in artificial phage DNA dimers (Hudnik-Plevnik & Bertani, 1980), after UV (*ibidem*), between repressed superinfecting phages (this paper), and with replication driven by a non-P2 replicon (*ibidem*).

These observations do not favor the hypothesis of a P2 encoded recombination inhibitor. They suggest rather that the low and *recA* independent frequency of recombination exhibited by lytically growing P2 is the consequence of peculiar characteristics of P2 replication and/or packaging mechanisms. It has become evident that a large variety of cellular functions are carried out by complex, machine-like macromolecular assemblies (see Alberts, 1998; Baker and Bell, 1998; Cook, 1999), often specifically localized in the cell. This view replaces the old naïve concept of an infected bacterial cell as a bag of enzymes with freely mixing viral nucleic acid molecules. In keeping with the new perspective, the unusually low recombination frequency of P2 might simply result from a difficulty for replicating P2 DNA in meeting other molecules derived from a different parent. This could be due to a very efficient encapsidation mechanism which entraps and wraps up every new DNA molecule as it is produced, and/or a very compact and sessile DNA replication mechanism where progeny DNA molecules are retained in a cluster, until the encapsidation process can begin. Various defined associations of replicating DNA with the cell membrane have been known for a long time (and demonstrated also for replicating P2 DNA: Ljungquist, 1973, 1976). Old investigations of phage replication by ordinary microscopy (Whitfield and Murray, 1954, for P2), in spite of the methodological efforts (see Robinow and Kellenberger, 1994), did not yield easily interpretable results. There have been since advances in optics and in labeling techniques. One of these, fluorescence *in situ* hybridization, has recently permitted the demonstration of localized clusters of replicating DNA for a plasmid (J. Pogliano and D. R. Helinski, personal communication) and could easily be applied to phage. If daughter copies of P2 DNA remained localized in the neighborhood of the parent particle, recombination between sisters could well occur at normal rates, but it would be genetically undetectable in standard crosses,

without resorting to special experimental arrangements. These suggestions would not be at odds with current ideas on the essential role of recombination in replication processes (see references cited in Introduction). On the other hand, the low level of recombination exhibited by lytically developing P2, being apparently invariant with the *recA* status of the host cell, could not simply be imputed to a few DNA copies escaping the above hypothesized constraints. Rather, it would have to result from an alternative, low efficiency, *recA*-independent recombination mechanism.

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**Footnote 1**

Throughout this section, recombination frequencies are given as frequencies of recombinants, i.e. they are based on the actual number of the *selected* recombinants, and are *not* multiplied by 2, as would be traditionally done to account for reciprocal recombinants.

**Footnote 2**

Several types of experiments were performed, all with negative results, and do not deserve to be presented in detail. They were: (a) allowing longer times for P2 to recombine during or prior to phage DNA replication (blocking protein synthesis with chloramphenicol, or delaying phage DNA synthesis of *tsA* mutants at the non-permissive temperature); (b) searching for P2 mutants sensitive to *recA* (i.e. able to grow on a *recA*<sup>-</sup> and not on a *recA*<sup>+</sup> host); (c) searching for P2 mutants that might recombine more frequently (by exploiting the tandem duplication mutant P2 *vir37* and looking for mutants showing a higher rate of resolution of the duplication); (d) searching for evidence of a P2 product that might inhibit recombination in another phage (crossing two  $\lambda$  *int red* phages in bacteria lysogenic for P2 *amL tsC5 del1*, at various times after the characteristically defective, i.e. unproductive, heat induction of the P2 lysogen); (e) testing for possible overproduction of DNA ligase in P2 infection (a P2 mutant was obtained that could scarcely grow in a ligase defective host, yet did not show increased recombination frequency); (f) searching for a possible effect of the *red* pathway of  $\lambda$  on P2 recombination (P2 crosses in  $\lambda$  lysogens, infected at various times after  $\lambda$  induction) or of increased *recA* product concentration (using the heat inducible mutant *tif* of *recA*); (g) P2 crosses where both parents were defective in gene *old* (and in site specific recombination). Experimental details of these attempts will be supplied upon request.

**Table 1** Strains used: properties, derivation, references

<u>Bacteriophages</u>	
P2 <i>amL302 del2 vir22 lg?</i>	From a UV cross P2 <i>amL302 tsD4 vir22 lg?</i> (of Bertani et al. 1978) × P2 <i>del2 lg</i> (of Bertani, 1975)
P2 <i>amL302 del6 tsC5 lg</i>	From a UV cross P2 <i>amL302 lg</i> (of Bertani et al. 1978) × P2 <i>del6 tsC5</i> (of LE Bertani, 1980)
P2 <i>amL302 del6 vir79 lg?</i>	From a cross P2 <i>amL302 tsD4 del6 vir79 lg?</i> × P2 <i>amL302 del6 tsC5 lg?</i>
P2 <i>amL302 tsD4 del6 vir79 lg?</i>	From a UV cross P2 <i>lg del6 vir79</i> × P2 <i>amL302 tsD4 int1 tsC5</i> . The latter originates from a series of crosses (not specified here for brevity sake) involving P2 <i>int1 tsC5</i> (of Nilsson and Bertani, 1977), P2 <i>vir79 amA127 old17</i> (of Bertani, 1975), P2 <i>tsA54</i> (of Lindahl, 1970) and P2 <i>lg am301</i> (unpublished).
P2 <i>del2 tsD4 vir22 lg?</i>	From a UV cross P2 <i>amL302 tsD4 vir22 lg?</i> (of Bertani et al. 1978) × P2 <i>del2 amA127 lg</i> . The latter is from a UV cross P2 <i>del2 lg</i> (of Bertani, 1975) × P2 <i>vir1 amA127 old17</i> (of Bertani, 1975)
P2 <i>del6 vir79 lg</i>	From a UV cross P2 <i>vir79 amA127 lg</i> (of Bertani, 1975) × P2 <i>del6</i> (of LE Bertani, 1980)
P2 <i>tsD4 del6</i>	LE Bertani (1980 and unpublished). Another isolate, from a UV cross P2 <i>del6</i> (of LE Bertani, 1980) × P2 <i>tsD4</i> (of Lindahl, 1969 a) was used in crosses 11 and 12, Table 2.
P2 <i>tsD4 del6 vir79 lg?</i>	From a cross P2 <i>amL302 tsD4 del6 vir79 lg?</i> × P2 <i>tsD4 del6</i>
P2/P4 Hy19 <i>amL302 lg?</i>	From a UV cross P2/P4 Hy19 <i>lg?</i> × P2 <i>amL302 del2 lg</i> (of Bertani et al. 1978)

P2/P4 Hy19 <i>lg?</i>	Lindqvist (1981 b). See Fig. 1. Unlike P2, and like P4, it grows on <i>rep</i> hosts.
P2/P4 Hy19 <i>tsD4 lg?</i>	From a UV cross P2/P4 Hy19 <i>lg?</i> × P2 <i>del2 tsD4 lg</i> (of Bertani et al. 1978)
P4 <i>del2<sub>4</sub></i>	P4 <i>del2</i> of Calendar et al. (1981). Symbol altered here to distinguish it from <i>del2</i> in P2.
P4 <i>del2<sub>4</sub> αam1 vir1</i>	From a cross P4 <i>del2<sub>4</sub></i> × P4 <i>αam1 vir1</i> . Methods as in Dehò (1983)
P4 <i>del2<sub>4</sub> εam104 vir1</i>	From a cross P4 <i>del2<sub>4</sub></i> × P4 <i>εam104 vir1</i> . Methods as in Dehò (1983)
P4 <i>αam1 vir1</i>	Gibbs et al. (1973)
P4 <i>εam104 vir1</i>	Geisselsoder et al. (1981)

Plasmids

pEE195	Derivative of the multicopy plasmid pBR322 with a P2 <i>Bgl</i> III fragment of about 620 bp, including the repressor gene C, inserted in the tetracycline resistance gene. It expresses C. (Westöö and Ljungquist, 1980).
pEE331	Same as pEE195, but carrying <i>tsC5</i> rather than <i>C<sup>+</sup></i> (E. Haggård-Ljungquist, unpublished)

Bacteria (all *Escherichia coli* strains)

C-1a	Sasaki and Bertani (1965).
C-1055	Wiman et al. (1970). Relevant properties: F <sup>+</sup> <i>str<sup>r</sup> sup<sup>+</sup></i> . Used as non-permissive indicator for P2.
C-1415	F <sup>-</sup> <i>rha his-4 rep-3</i> (Calendar et al. 1970)
C-1757	Sunshine et al. (1971). Relevant properties: <i>str<sup>r</sup> supD</i> . Used as permissive indicator for P2.
C-1844	Sunshine et al. (1971). Relevant properties: <i>str<sup>r</sup> suA</i> . Used as non-permissive, polarity suppressing indicator for P2

C-2091	<i>his supD</i> (LE Bertani, 1980)
C-2898	<i>F rha rep-3 supD</i> . From C-1415 by transduction with phage P1 grown on C-1757, selecting for <i>his</i> <sup>+</sup>
C-5204	Dehò (1983). Relevant properties: <i>F</i> <sup>+</sup> (P2) <i>sup</i> <sup>+</sup> <i>str</i> <sup>r</sup>
C-5205	Dehò (1983). Relevant properties: <i>F</i> <sup>+</sup> (P2) <i>supD str</i> <sup>r</sup>
CK-104	<i>F recA13</i> . A hybrid between <i>E. coli</i> C and a K12 <i>recA13</i> Hfr strain (no. 12 of Hertman, 1967) isolated by L. E. Bertani (unpublished)
K-221	<i>F endAI supE44 thi-1 λ<sup>r</sup> his-211 hsd-16</i> (Hudnik-Plevnik and Bertani, 1980)
K-234	<i>F endAI supE44 thi-1 λ<sup>r</sup> his-211 hsd-16 recA13</i> (Hudnik-Plevnik and Bertani, 1980)
K-254	<i>Mal</i> <sup>+</sup> <i>λ</i> <sup>s</sup> revertant of K-221
K-256	<i>Mal</i> <sup>+</sup> <i>λ</i> <sup>s</sup> revertant of K-234
K-287	K-254 carrying plasmid pEE331
K-288	K-256 carrying plasmid pEE331
K-289	K-221 carrying plasmid pEE195
K-290	K-234 carrying plasmid pEE195
KG-61	K-221 made lysogenic for P2
KG-62	K-234 made lysogenic for P2

### Abbreviations

*am*, suppressor sensitive mutation; *C*, immunity repressor gene in P2; *D*, a tail gene in P2; *del19*, deletion that arose in the process of isolation of P2/P4 Hy19 (Lindqvist, 1981b), approximately coextensive with *vir22*; *del2*, a P2 deletion; *del24*, in P4, integration defective deletion; *del6*, integration defective deletion in P2; *L*, head maturation gene in P2; *Ig*, in P2, temperature dependent larger plaque; *ts*, temperature sensitive mutation; *vir1*, homonymous mutations: in P2, unable to lysogenize, but sensitive to immunity, and in P4, partially insensitive to immunity; *vir22* and *vir79*, in P2, immunity insensitive deletions;  $\alpha$ , P4 replicase/primase gene;  $\epsilon$ , P4 gene for derepression of helper prophage.

For each phage strain the mutation symbols (with the exception of *lg*) are listed in map order. The presence of the *lg* mutation (Bertani et al. 1969, 1978) is difficult to demonstrate in certain marker combinations. In the Table, *lg?* indicates the possible presence of this character as a consequence of the genealogy of the strain. Elsewhere in this paper the presence or absence of *lg* is ignored.

For bacterial gene symbols, see Bachmann (1990).

**Table 2** Dependence of recombination frequency on the *recA* status of the host bacterium

Cross	MOI <sup>a</sup>	Parent phages	Selected type <sup>b</sup>	Recombinant frequency in <i>recA</i> host	Recombinant frequency <sup>c</sup> in <i>recA</i> <sup>+</sup> host	Ratio <sup>d</sup>	
1	10	P4 $\alpha$ am1 + <i>vir1</i>	$\alpha^+ \epsilon^+$	KG-62	KG-61	$7.5 \times 10^{-4}$	$107 \times$
	10	P4 + $\epsilon$ am104 <i>vir1</i>					
2	10	P4 <i>del24</i> $\alpha$ am1 + <i>vir1</i>	$\alpha^+ \epsilon^+$	KG-62	KG-61	$2.2 \times 10^{-4}$	$\geq 122 \times$
	10	P4 <i>del24</i> + $\epsilon$ am104 <i>vir1</i>					
3	6	P2 <i>amL302 del2</i> + <i>vir22</i>	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>	K-234	K-221	$(4 \times 10^{-4})$	
	6	P2 + <i>del2 tsD4 vir22</i>					
4	8	P2/P4 Hy19 <i>amL302</i> +	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>	K-234	K-221	$1.6 \times 10^{-2}$	$\geq 89 \times$
	4	P2/P4 Hy19 + <i>tsD4</i>					
5	8, 8	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>	CK-104	C-2898	$2.3 \times 10^{-3}$	$56 \times$
6	10, 8	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>	CK-104	C-1a	$1.4 \times 10^{-2}$	$64 \times$
7	10, 8	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>		C-2898	$3.1 \times 10^{-3}$	
8	10, 8	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>		C-2091	$2.0 \times 10^{-2}$	
9	10, 8	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>		C-1415	$9.6 \times 10^{-3}$	
10 <sup>e</sup>	7	P2 <i>amL302</i> + <i>del6 vir79</i>	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>	K-290	K-289	$4.1 \times 10^{-4}$	$1.1 \times$
	7	P2 + <i>tsD4 del6 vir79</i>					
11 <sup>f</sup>	6	P2 <i>amL302</i> + <i>del6 tsC5</i>	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>		K-221	$1.0 \times 10^{-3}$	
	6	P2 + <i>tsD4 del6</i> +					
12	5, 5	as above	<i>am</i> <sup>+</sup> <i>ts</i> <sup>+</sup>		K-221	$1.3 \times 10^{-3}$	

<sup>a</sup> Approximate multiplicities of infection.

<sup>b</sup> For crosses 1 and 2, see Materials and methods. For the other crosses, selection was at 42° to 43°C, with amber-non-permissive indicators C-1844 (cross 3), C-1415 (crosses 4 through 9), and C-1055 (crosses 10 through 12). Non-selective assays (at 30°C) used C-1757 (crosses 3, 10 through 12) and C-2898 (crosses 4 through 9).

<sup>c</sup> Number of plaques of selected type divided by total phage yield in cross. Data in parentheses are based on small recombinant plaque numbers. For other properties of the host bacteria used see Table 1.

<sup>d</sup> Recombinant frequency in *recA*<sup>+</sup> host divided by the corresponding frequency in *recA* host.

<sup>e</sup> Ampicillin present as in crosses of Table 3. Incubation at 30°C throughout. Burst sizes: 58 in K-290, 154 in K-289.

<sup>f</sup> Unlike all other experiments, broth without added glucose was used here.

**Table 3** Recombination of P2 in the preprophage state: frequencies of selected recombinant types

Experiment <sup>a</sup>	1	2	3	4	5	6	7	8	9 <sup>b</sup>	10 <sup>b</sup>	11 <sup>c</sup>
Host bacteria	K-289	K-289	K-289	K-290	K-287	K-287	K-287	K-288	K-287	K-287	K-287
<i>recA</i> status	+	+	+	-	+	+	+	-	+	+	+
Gene C allele in plasmid	+	+	+	+	<i>tsC5</i>	<i>tsC5</i>	<i>tsC5</i>	<i>tsC5</i>	<i>tsC5</i>	<i>tsC5</i>	<i>tsC5</i>
<u>Infection I<sup>a</sup>:</u>											
Dilution after infection	200×	400×	2,000×	200×	200×	400×	2,000×	200×	200×	200×	200×
Incubation time (min) <sup>b</sup>	260	290	380	405	240	270	360	360	240	240	180
Bacterial multiplication factor <sup>c</sup>	21.5×	39×	175×	9.5×	29×	54×	208×	20.5×	27.5×	28×	34×
Expected preprophages per bacterium at Infection II <sup>d</sup>	0.43	0.24	0.05	0.97	0.32	0.17	0.04	0.45	0.34	0.33	0.27
<u>Infection II<sup>a</sup>:</u>											
Midwife phage, multiplicity of infection	2.4	2.4	2.4	2.4	2.0	2.0	2	2	2	2	2.4
Average burst size <sup>e</sup>	137	112	122	37	77	71	60	14	45	50	58
<i>am<sup>+</sup> ts<sup>+</sup></i> phage in progeny calculated per ml of infection II mix <sup>f</sup>	4.7×10 <sup>4</sup>	2.4×10 <sup>4</sup>	4.8×10 <sup>3</sup>	9.2×10 <sup>2</sup>	3.0×10 <sup>4</sup>	1.9×10 <sup>4</sup>	7.5×10 <sup>3</sup>	<10 <sup>2</sup>	<5×10 <sup>2</sup>	<5×10 <sup>2</sup>	2.3×10 <sup>4</sup>
Same, as fraction of expected preprophages	2.4×10 <sup>-4</sup>	2.3×10 <sup>-4</sup>	2.0×10 <sup>-4</sup>	2.1×10 <sup>-6</sup>	2.0×10 <sup>-4</sup>	2.5×10 <sup>-4</sup>	3.8×10 <sup>-4</sup>	<5×10 <sup>-7</sup>	<3×10 <sup>-6</sup>	<3×10 <sup>-6</sup>	2.1×10 <sup>-4</sup>
Same, actual plaques scored	358	101	21	13	276	94	34	0	0	0	881

<sup>a</sup> The host bacteria carried a plasmid (either pEE195 or pEE331) expressing the P2 repressor gene C (either the wild type or the temperature sensitive *tsC5*). Throughout the experiment, growth was at 30°C, in broth with ampicillin (10 µg/ml, to select against plasmid loss) and CaCl<sub>2</sub> (2.5 mM, transiently raised to 5 mM in the adsorption mixtures I and II). Aerated cultures grown to between 4 and 7 × 10<sup>7</sup>/ml were concentrated by centrifugation to 5 × 10<sup>8</sup>/ml. In experiments 1 through 8, and 11, a 0.8 ml sample of the suspension was mixed with the two immunity sensitive phages P2 *tsD4 del6* and P2 *amL302 del6 tsC5*, prepared in a 0.2 ml volume, at a 1:1 ratio (Infection I, time 0). Experiments 9 and 10 are single parent phage controls. The total multiplicity of infection was 9.2, after correction for unabsorbed phage (approximately 15% of the input, apparently unaffected by the *mal* status of the bacteria) measured at 45 min (30 min in experiment 11). At these times the mixture was diluted at least 200-fold, in 20 ml prewarmed broth and incubated further. When this culture approached a bacterial titer of 4 × 10<sup>7</sup>/ml, it was placed on ice and stored overnight. On the following day, bacteria were counted and the culture was centrifuged and resuspended at 5 × 10<sup>8</sup>/ml and then infected with the midwife phage P2 *amL302 tsD4 del6 vir79* (Infection II, new time 0, multiplicity of infection between 2.0 and 2.4). After 20 min for adsorption, this mixture was diluted at least 100-fold and incubated for 100 to 120 additional minutes, at which time it was placed on ice and assayed: for total phage at 30°C on the amber-suppressor strain C-1757, and for *am<sup>+</sup> ts<sup>+</sup>* recombinants at 42°C to 43°C on the non-permissive indicator strain C-1055. Both indicators are streptomycin resistant, and streptomycin was present in all plates in order to inactivate any bacteria that survived Infection II. Alternatively (experiment 11), the lysate was filtered through bacteria-retaining membrane filters prior to the assays.

<sup>b</sup> Includes the adsorption period at high bacterial concentration.

<sup>c</sup> Based on the total bacterial count in the culture stored overnight, just before concentration for Infection II, and on the original input in Infection I. The generation times (assuming, in first approximation, no lag during or following Infection I) would be on average 53 minutes for *recA<sup>+</sup>* strains, much longer for the *recA<sup>-</sup>* strains (83 minutes for K-288, 125 minutes for K-290).

<sup>d</sup> Calculated by dividing the average number of adsorbed phages per bacterium in Infection I by the bacterial multiplication factor. It is not corrected for the loss resulting from the small proportion of superinfected bacteria that lysed prior to Infection II (see under (a) in the

Results section). Assuming that the bacteria that lysed were the ones receiving the highest numbers of superinfecting phage particles, the estimates given on this line would be in excess by at most 5%.

<sup>e</sup> Total phage produced (plaques at 30°C, on permissive indicator) divided by the calculated number of bacteria infected with at least one particle of midwife phage.

<sup>f</sup> Based on the total number of plaques (both turbid and clear, i.e.  $C^+$  and  $tsC5$ ) obtained at 42°C on the non-permissive indicator. In experiments 1 through 8, removal of the supernatant when bacteria were concentrated by centrifugation in preparation for Infection II eliminated most of the phage present in the cultures (see under (a) in the Results section) prior to Infection II. Based on parallel phage titrations (not given in the Table) of the supernatants, one can calculate that with  $recA^+$  hosts the contribution of  $am^+ ts^+$  phage carried over with the bacterial pellet would be less than 1/100 of the corresponding values given in this row. With  $recA^-$  hosts (experiments 4 and 8) the calculated carry over was at the same level as the observed values after Infection II. The estimate in experiment 11 does include  $am^+ ts^+$  phage present prior to Infection II, to the extent of about 16% of the total given here.

<sup>g</sup> Control: only phage P2  $tsD4 del6$  (at multiplicity of infection about 9) was used for Infection I.

<sup>h</sup> Control: only phage P2  $amL302 del6 tsC5$  (at multiplicity of infection about 9) was used for Infection I.

<sup>i</sup> At variance from the rest of the Table, for experiment 11, 20 (rather than 10) µg/ml ampicillin were used, and 5 mM  $MgCl_2$  were added to the medium. Also, on concentrating the superinfected cultures for Infection II, the bacteria were here resuspended in their own supernatant, and the recombinant data from Infection II thus include any recombinant phage preexisting Infection II.

**Table 4** Segregation of unselected markers

Part 1		Cross:		P2 <i>amL302</i> + <i>del6 tsC5</i>		P2 + <i>tsD4 del6</i> +	
Experiment	Plasmid type	<i>am<sup>+</sup> ts<sup>+</sup></i> progeny that was <sup>a</sup>		<i>tsC5</i>		<i>C<sup>+</sup></i>	
A. Expected for unbiased exchange distribution <sup>b</sup>						6.3 to 14.	
B. Recombination in the prephage state							
Experiments 1, 2, and 3 from Table 3 (pooled)	<i>C<sup>+</sup></i>	444	36	(0)	7.5		
Experiments 5, 6, and 7 from Table 3 (pooled)	<i>tsC5</i>	378	26	(3)	6.4		
Experiment 11 from Table 3 <sup>c</sup>	<i>tsC5</i>	675 <sup>c</sup>	32 <sup>c</sup>	(8)	4.5		
Additional experiment <sup>d</sup>	<i>tsC5</i>	369	46	(0)	11.1		
C. Recombination in standard mixed infection							
Experiment 11 from Table 2	none	1835	13	(0)	0.70		
Experiment 12 from Table 2	none	3916	16	(2)	0.41		
<b>Part 2</b> (data from Table 5, crosses 31 and 32, of Bertani, 1975) (standard mixed infection)							
Cross <sup>e</sup>	<i>ts<sup>+</sup> am<sup>+</sup></i> progeny that was		Percent exchange in <i>ori</i> containing arc		Percent expected for unbiased exchange distribution <sup>f</sup>		
	<i>del1</i>	<i>del<sup>+</sup></i>					
<i>tsD4 int1</i> + + + <i>int1 amA127 del1</i>	37	53	41.1		10.		
<i>tsD4 int1</i> + <i>del1</i> + <i>int1 amA127</i> +	44	24	35.3		10.		

<sup>a</sup> The classification of *C<sup>+</sup>* vs. *tsC5* plaques was done by inspection of the selective plates (C-1055 indicator at 42°C). Suspected *C<sup>+</sup>* or mixed plaques were picked and progeny tested. In addition to

the typical, homogeneously turbid  $C^+$  plaques, a small number of mottled  $C^+/tsC5$  plaques were observed. These were included among the  $C^+$  types (their number being given in parentheses) when, upon progeny testing, the proportions of  $C^+$  types was 30% or higher. We tentatively attribute these plaques to the presence of Hershey heterozygotes (see Doermann et al. 1955). They don't seem to be evenly distributed among the various experiments. We cannot exclude scoring biases different for different experiments, depending on minor differences in plating conditions over time and on the manner of progeny testing. A total of 70 plaques classified as  $tsC5$  were also picked and tested at 30°C: none was *vir79* rather than  $tsC5$ .

<sup>b</sup> Length of the shorter arc *tsD4* to *tsC5* (approximately 1.8 kilobases) as percentage of the arc *tsD4* (over *ori* and *cos*) to *amL302* (approximately 14 kilobases), with the length of *del6* subtracted (see Fig. 1). Since the exact locations of mutations *tsD4* and *amL302* within the respective genes D and L have not been established, the calculated percentage is given as a range that encompasses all possible location combinations within the two genes.

<sup>c</sup> The data on this line are corrected for the presence of recombinant phage preexisting Infection II (see note <sup>(i)</sup> under Table 3) based on the following data. The original score was 847  $tsC5$  and 34  $C^+$ . The corresponding score among recombinants preexisting Infection II (see (a) and (b) in the Results section) was 659  $tsC5$  and 8  $C^+$ . Total recombinants (calculated per ml of Infection II adsorption mix) were  $2.3 \times 10^4$  (Table 3) and the corresponding titer prior to Infection II was  $3.7 \times 10^3$ .

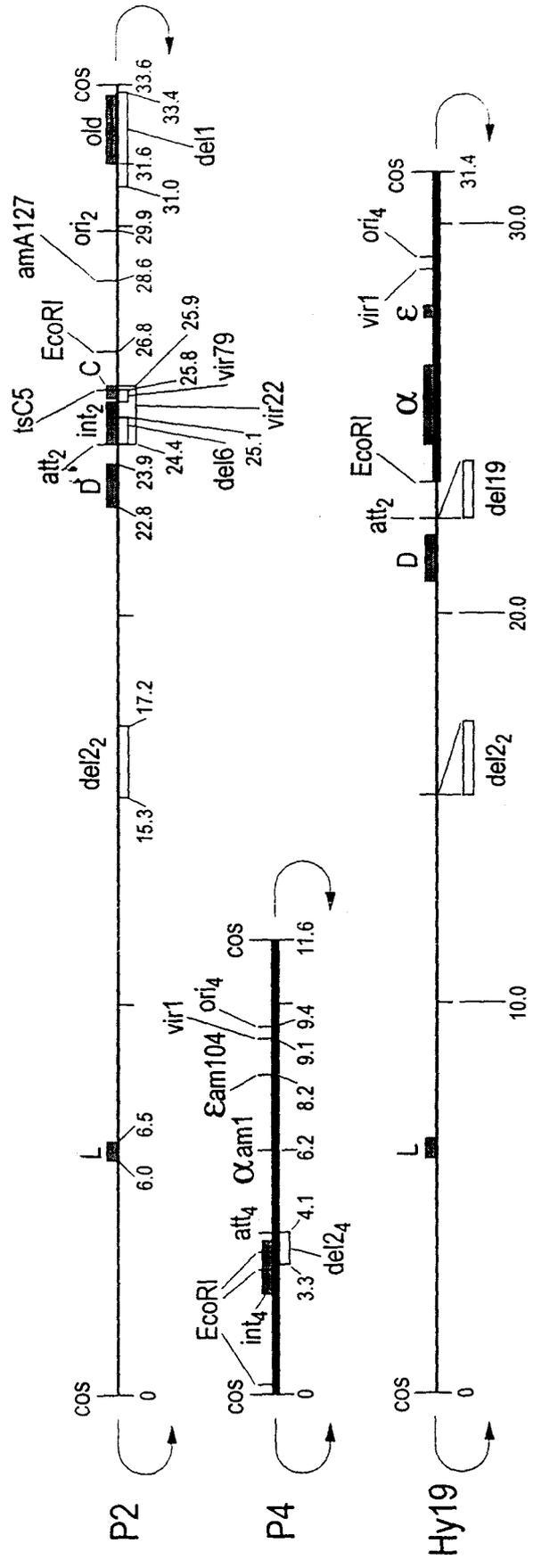
<sup>d</sup> This was exactly like experiments 5, 6 and 7 in Table 3, except that the dilutions after Infection I were 6× and 200×, and the bacterial multiplication factors were respectively 9.5× and 16.5×.

<sup>e</sup> Standard conditions. Host: C-1757. Burst sizes: 110 and 120.

<sup>f</sup> Ratio of arc *amA127* (over *ori*) to *del1* to arc *amA127* (over *ori* and *cos*) to *tsD4* (see Fig. 1), with the length of *del1* subtracted..

## Legend to Figure 1

Fig. 1. DNA maps of P2, P4 and P2/P4 Hy19, showing the elements relevant to this work. Distances are from the conventional left ends and are given in kbp. Data for P2 and P4 are from GenBank accession numbers AF063097 and X51522, respectively. The map for P2/P4 Hy19 is based on Lindqvist's (1981b) construction which joined the P2 *EcoRI* restriction site at 26.8 kbp and one of the two P4 *EcoRI* sites within the *int* gene, most probably the rightmost one. The curved arrows at the ends are to remind the reader that all three structures are circular when not encapsidated. For symbols, see end note in Table 1. Subscripts 2 and 4 refer to P2 and P4, respectively, and are used here for clarity where needed. The exact positions of mutations *tsD4* and *amL302* within D and L have not been determined. Mutation *tsC5* is not covered by the *vir79* deletion.



Bertani & Deho Fig. 1