Low-Frequency Rescue of a Genetic Marker in Deoxyribonucleic Acid from *Bacillus* Bacteriophage ϕ 105 by Superinfecting Bacteriophage¹

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Markers in gene L, which maps at the right end of the vegetative and prophage maps, are rescued at a strongly reduced frequency from mature ϕ 105 deoxyribonucleic acid (DNA) by superinfecting phage but at high frequency from vegetative and prophage DNA. It is suggested that the ends of mature DNA are degraded when DNA is taken up by competent cells.

Deoxyribonucleic acid (DNA) extracted from purified ϕ 105 phage gives one infectious center per 10^7 to 10^8 molecules added to competent cells, whereas single markers are rescued at about a 10^4 -fold higher frequency (10). When isolating suppressor-sensitive (*sus*) mutants of ϕ 105, we happened to identify a gene which rescues from mature DNA at a strongly reduced frequency compared to all other markers tested. In this paper, we will describe the properties of this gene in marker rescue and its relation to the structure and infectivity of mature ϕ 105 DNA.

MATERIALS AND METHODS

The bacteria and phage employed and the general methods used are described in the preceding paper (2). In one experiment, DNA was released from ϕ 105 particles by NaClO_4 as described by Freifelder (6).

RESULTS

Mapping of *sus2*, *sus9*, and *sus10*. Mutations *sus2*, *sus9*, and *sus10* are closely linked and fail to complement each other when mixedly infecting *su*⁻ bacteria (Table 1). They belong to complementation group (gene) L, which maps at the extreme right end of the vegetative as well as the prophage map (2).

Rescue of the wild-type allele of gene L from ϕ 105 DNA. Mature wild type ϕ 105 DNA was added to competent *su*⁺ bacteria. After uptake of DNA, the bacteria were superinfected with genetically labeled phage and the complexes were plated on *su*⁻ indicator. *Sus2*, *sus9*, and *sus10* rescue at about a 50-fold lower frequency compared to the other markers employed (Table 2).

The poor rescue of *sus2* is not related to the function of gene L since (i) the experiments were done in a *su*⁺ host, and (ii) in a *ts-sus2* double mutant the *ts*-marker rescues at a high frequency whereas *sus2* does not.

We have previously shown that infectious prophage, vegetative, and mature ϕ 105 DNA, respectively, sediment at different rates in neutral sucrose, demonstrating size, conformational differences, or both, between these DNA species (11). It might be expected that the L gene would rescue at a high frequency from prophage or vegetative DNA compared to mature DNA. As seen from the results shown in Table 3 this is indeed the case. We can thus conclude that poor rescue of markers in gene L does not depend on the nature of the mutation, i.e., the type of base pair change, but rather reflects a structural characteristic of mature DNA.

Where is gene L lost for rescue? There are three main steps in the rescue experiment where gene L may be preferentially affected. (i) The L gene could be lost during DNA extraction. (ii) Due to its position at one end of the DNA molecule, the L gene might rarely become integrated into the DNA of the superinfecting phage if rescue occurs by an even number of exchanges between two linear molecules. (iii) The L gene might be lost for rescue during binding of DNA by competent cells.

We have not been able to design a direct experiment to test for loss of gene L at extraction of DNA. The following experiments pertinent to this question have been performed, however. The L gene rescues equally poorly from mature DNA released by NaClO_4 as from phenol-extracted DNA (Table 4). Generating new ends on the

¹ Parts of this work were presented at the 10th International Congress for Microbiology, 9-15 August 1970, Mexico City.

TABLE 1. Two-factor crosses and complementation with mutations in gene *L*^a

Cross	Per cent recombination	Per cent complementation
<i>sus2</i> × <i>sus9</i>	0.7	<0.2
<i>sus2</i> × <i>sus10</i>	0.8	<0.2
<i>sus2</i> × <i>sus7</i> (K)	3.0	25
<i>sus2</i> × <i>tsN31</i> (K)	6.1	
<i>sus2</i> × <i>sus11</i> (J)	7.6	
<i>sus2</i> × <i>tsN34</i> (H)	9.2	
<i>sus9</i> × <i>sus7</i> (K)		23
<i>sus10</i> × <i>sus7</i> (K)		31

^a Crosses were performed in SR135 (*su*⁺) at 30 C. Complementation was done in 168 (*su*⁻) at 41 C; the results are expressed as per cent of wild-type phage burst size.

TABLE 2. Marker rescue of gene *L* and other genes from mature $\phi 105$ DNA^a

Superinfecting phage	DNA preparation	Infectious centers ^b	
		<i>su</i> ⁻ 30 C	<i>su</i> ⁺ 40 C
<i>sus2</i>	1	0.1	
<i>sus9</i>	1	0.01	
<i>sus10</i>	1	0.3	
<i>sus19</i>	1	13	
<i>tsN15</i> — <i>sus19</i>	2	31	22
<i>tsN15</i> — <i>sus14</i>	2	25	22
<i>tsN15</i> — <i>sus13</i>	2	22	20
<i>tsN15</i> — <i>sus12</i>	2	25	22
<i>tsN15</i> — <i>sus11</i>	2	19	25
<i>tsN15</i> — <i>sus7</i>	2	6.3	7
<i>tsN15</i> — <i>sus2</i>	2	0.5	17
<i>tsN15</i> — <i>sus2</i>	3	0.7	37
<i>tsN31</i> — <i>sus2</i>	3	1.3	33

^a Experiments were performed in competent *su*⁺ bacteria, and the complexes were plated under conditions restrictive for *sus* mutants (*su*⁻ 30 C) or *ts* mutants (*su*⁺ 40 C). DNA was used at a final concentration of 0.01 μ g/ml.

^b Values expressed $\times 10^{-4}$.

DNA molecule by shearing does not improve or impair rescue of gene *L* (Table 5). If gene *L* is taken up intact by competent cells but yet rescues poorly, its presence in those cells might be detected if it could complement a superinfecting phage carrying a mutation in gene *L*. However, attempts to demonstrate complementation between mature DNA and superinfecting phage have been unsuccessful (Table 6).

To test whether gene *L* has a lower probability of being rescued due to the recombinational mechanism involved, the following experiment was done. Wild type $\phi 105$ was irradiated with ultraviolet (UV), and competent *su*⁺ bacteria

TABLE 3. Marker rescue from prophage and vegetative $\phi 105$ DNA^a

Superinfecting phage	Type of DNA	Infectious centers ^b
<i>sus2</i>	Pro	2.2
<i>sus13</i>	Pro	2.5
<i>sus19</i>	Pro	1.6
None	Pro	0.01
<i>sus2</i>	Veg	15
<i>sus19</i>	Veg	15
None	Veg	0.05
<i>sus2</i>	VegS	3.1
<i>sus19</i>	VegS	2.9
None	VegS	<0.001

^a Experiment was performed in competent *su*⁺ bacteria and infectious centers were assayed on on *su*⁻ bacteria at 30 C. About 0.1 μ g of DNA per ml was used. Pro = prophage DNA; veg = vegetative DNA; vegS = vegetative DNA sheared under conditions that yield predominantly half molecules from mature $\phi 105$ DNA.

^b Values expressed $\times 10^{-4}$.

TABLE 4. Marker rescue from mature $\phi 105$ DNA released by treatment of whole phage with NaClO₄^a

Phage preparation	Infectious centers	
	<i>su</i> ⁻ 30 C	<i>su</i> ⁺ 40 C
I — Deoxyribonuclease	1.2×10^3	5.9×10^4
I + Deoxyribonuclease	<10 ²	<10 ²
II — Deoxyribonuclease	2.4×10^3	9.1×10^4
II + Deoxyribonuclease	<10 ²	<10 ²

^a About 2×10^{10} PFU in 0.1 ml were added to 1 ml of 7.5 M NaClO₄ (pH 7.5) at room temperature. After 15 min, 0.1 ml was added to 1 ml of competent *su*⁺ bacteria in two tubes; one tube contained 50 μ g of pancreatic deoxyribonuclease (Worthington Biochemical Corp., Freehold, N.J.). Twenty minutes later, the bacteria were superinfected with $\phi 105$ *tsN15* — *sus2* and the complexes were plated under conditions restrictive for growth of either *ts* or *sus* mutants. Phage preparation I was concentrated from an MC-induced lysate of 168 ($\phi 105$) by one cycle of low- and high-speed centrifugation. Phage preparation II was the same lysate further purified by banding in CsCl.

were infected with irradiated phage at low multiplicity. The bacteria were then superinfected with genetically labeled phage at high multiplicity and the complexes were plated on *su*⁻ bacteria. Gene *L* was found to rescue as well as gene *C* (*sus19*) from UV-inactivated phage (Table 7). Thus, gene *L* is rescued at a high frequency from a DNA

TABLE 5. Marker rescue from whole and sheared mature $\phi 105$ DNA^a

Superinfecting phage	Infectious centers	
	Whole DNA	Sheared DNA
<i>sus2</i>	1.2×10^4	3.7×10^3
<i>sus19</i>	6.3×10^5	1.9×10^5

^a Mature $\phi 105$ DNA, labeled with ^3H -thymidine, was sheared in a Sorvall Omnimixer as described (2). Analysis by sedimentation in neutral sucrose (11) indicated at least 80% half molecules in the preparation; 0.1 μg of DNA was added per ml of competent *su*⁺ bacteria.

TABLE 6. Lack of complementation between mature $\phi 105$ DNA and superinfecting phage in competent *su*⁻ bacteria^a

Superinfecting phage	Infectious centers		
	<i>su</i> ⁻ 30 C	<i>su</i> ⁺ 40 C	<i>su</i> ⁻ 40 C
<i>tsN15</i> — <i>sus2</i>	7×10^2	$<10^2$	$<10^2$
<i>tsN15</i> — <i>sus12</i>	1.9×10^4	1.5×10^3	3.0×10^3
<i>tsN9</i> — <i>sus11</i>	1.4×10^4	1.6×10^3	1.0×10^3
<i>tsN31</i> — <i>sus19</i>	4.1×10^4	1.8×10^3	2.3×10^3

^a To 1 ml of competent *su*⁻ bacteria was added 0.01 μg of mature $\phi 105$ DNA. Twenty minutes later, the bacteria were superinfected with *ts* — *sus* double mutants, and the complexes were plated under conditions nonpermissive for growth of the one type or both types of mutants.

TABLE 7. Marker rescue from ultraviolet (UV)-inactivated $\phi 105$ phage^a

Superinfecting phage	No. of lethal UV hits	Infectious centers <i>su</i> ⁺
<i>sus2</i>	9	15
<i>sus19</i>	9	20
<i>sus2</i>	36	3.4
<i>sus19</i>	36	4.1

^a Values expressed $\times 10^{-4}$.

^b Wild type $\phi 105$ was irradiated with UV and used to infect competent *su*⁺ bacteria at an MOI of total phage of about 0.05. Ten minutes later, the cells were superinfected with unirradiated, genetically labeled phage and the complexes were plated under conditions nonpermissive for growth of the superinfecting phage.

molecule unable to initiate a productive infection when this molecule is introduced into the cell by the phage "injection" mechanism, but not when it is taken up by the mechanism employed by competent bacteria.

In phage SP82G, marker rescue occurs at maximal frequency when the superinfecting phage is added before DNA (7); preinfection is thought to minimize destruction of added DNA by bacterial enzymes. Little increase in the relative rescue of gene L was found when superinfecting phage was added to competent cells at various times before addition of DNA (Table 8).

Rescue of other markers. The wild-type allele of *sus7* of gene K is often rescued at a reduced frequency from mature $\phi 105$ DNA. This probably reflects a property of phage carrying this mutation since in a double mutant *N15-sus7* both markers rescue at the same reduced frequency (Table 2). Furthermore, *tsN31*, which is also located in gene K, rescues at a normal high frequency.

DISCUSSION

There are two obvious conclusions to be drawn from the above experiments (i) All markers in mature ϕ DNA are not rescued at equal frequencies. (ii) The low rescue observed for gene L reflects a structural difference between, on the one hand, mature DNA and, on the other hand, prophage and vegetative DNA.

We have not been able to exclude the possibility that gene L is lost during extraction of mature DNA. This alternative is not very attractive, though, for the following reasons. (i) The ratio of rescue of *sus2* and *tsN15* is essentially constant in three different DNA preparations extracted with phenol; extraction of DNA by NaClO_4 does not affect rescue of gene L. (ii) The residual rescue activity is no more susceptible to shearing forces than other markers, nor is gene L more susceptible to UV. (iii) Small DNA pieces have not been seen after ultracentrifugation or electron microscopy of mature DNA, as described for DNA from $\phi 29$ (1).

TABLE 8. Marker rescue from preinfected competent *su*⁺ bacteria exposed to mature $\phi 105$ DNA^a

Time of infection (min)	Infectious centers ^b	
	<i>sus2</i>	<i>sus19</i>
+ 15	0.6	13
+ 10	1.1	15
0	0.6	8.3
- 20	0.3	11

^a Superinfecting phage was added to competent *su*⁺ bacteria at various times before (+) or after (-) addition of 0.01 μg of mature $\phi 105$ DNA. Ten minutes after addition of DNA (or superinfecting phage at -20 min), the complexes were plated on *su*⁻ indicator.

^b Values expressed $\times 10^{-4}$.

The fact that gene L rescues at a high frequency from UV-inactivated whole phage seemingly rules out the possibility that it rescues poorly from mature DNA because of some particular feature of the recombinational mechanism employed in marker rescue. Binding of DNA to a structure unique to the competent cell (13) cannot per se explain our results since gene L rescues well from prophage and vegetative DNA. We are thus led to believe that gene L is selectively affected during some stage of binding of mature DNA by competent bacteria in a manner such that it rescues at a reduced frequency.

The basis for the low rescue of gene L must be found in the structure of mature DNA at or close to this gene. The results of vegetative crosses place gene L at the extreme right end of the map (2). The ends may be terminated by a single-stranded region. The only published evidence supporting this suggestion is the finding of apparently circular molecules in electron micrographs of $\phi 105$ DNA (3). We are currently exploring the end structure of $\phi 105$ DNA with the aid of DNA polymerase and exonucleases of defined specificity (8).

It has been suggested by several authors that during some stage of uptake or integration (or both) of DNA in competent bacteria (part of the molecule becomes single-stranded (4). The biological activity of single-stranded DNA is very low under the conditions employed by us, probably due to degradation of such DNA (5, 12). When a DNA molecule with single-stranded ends is bound by competent cells, these ends may become degraded, and degradation may continue into the double-stranded part of the molecule. If the L gene is located at the end of the mature DNA molecule (assuming that rescue occurs only between double-stranded molecules), the poor rescue of gene L can be accounted for. An alternative possibility is that only the single strands (which should then be complementary) are degraded. This will prevent the DNA molecule from circularizing after being taken up by a competent cell, and rescue will occur between one linear and one linear or circular molecule. If rescue occurs by an even number of exchanges between the two molecules a terminal marker would rescue at a reduced frequency. This alternative is difficult, however, to reconcile

with the normal rescue of gene L from prophage DNA.

The data strongly suggest that gene L occupies a unique position on the mature DNA molecule; the gene sequence can thus not be randomly permuted. The above proposals also offer an explanation for the low infectivity of mature $\phi 105$ DNA.

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