Mapping of Prophage and Mature Deoxyribonucleic Acid from Temperate *Bacillus* Bacteriophage ϕ 105 by Marker Rescue

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By using temperature-sensitive (*ts*) and suppressor-sensitive (*sus*) mutants, 11 essential genes have been identified in phage ϕ 105. The order of the genes has been established in two- and three-factor crosses. The genes can be arranged in a linear order; this order is identical in the vegetative phage and in the prophage. One gene essential for phage deoxyribonucleic acid (DNA) synthesis has been found. Marker rescue from prophage and mature DNA, taken up by competent bacteria, was studied by superinfection with phage carrying one *sus* and one *ts* mutation. In prophage DNA, all single markers studied are rescued at similar frequencies. The frequency of co-rescue of two markers is proportional to the recombinational distance between the markers. Thus, colinearity between the genetic map and the position on the DNA molecule of those mutations used to establish the map is demonstrated. The results indicate that the recombination frequencies observed in vegetative crosses are a relative measure of the physical distance between markers. All single markers are not rescued at equal frequencies from mature DNA. The frequency of co-rescue of two markers is related to the recombinational distance only over a distance about one-fourth or less of the genetic map. Markers separated by 10% recombination, or more, are co-rescued at 5 to 10% of the frequency of rescue of single markers. Shearing of mature DNA into half-sized molecules reduces the efficiency by which single markers are rescued by a factor of 5 to 10. The results of experiments on co-rescue of two markers from half-sized mature DNA indicate a preferred break-point near the middle of the genetic map; the results are compatible with a nonpermuted sequence in mature DNA. It is pointed out and discussed that mature DNA exhibits several anomalies in marker rescue experiments.

Systems which allow experiments on the biological activity of purified viral nucleic acids are of particular importance for studies on the structural and functional organization of viral genomes. The activity of the whole genome can be assayed as the infectivity of a given nucleic acid preparation, or the presence of single genes can be detected by marker rescue (8, 19). By studying the distribution of markers in whole and fragmented deoxyribonucleic acid (DNA), for example, the existence of a permuted or unique gene sequence can be examined, and some estimate of the physical distances separating genetic markers may be obtained.

The helper phage assay of Kaiser and Hogness (12) has been used to study the organization of phage lambda DNA. Only molecules possessing intact single-stranded ends are active in this assay (22). This fact has been used to establish a unique gene order in lambda DNA as well as

to estimate the physical distance between various markers at the end of the molecule (13, 22). Free single-stranded ends are not essential for the infectivity of lambda DNA in other types of assays (24). In *Bacillus* bacteriophage SP82G, marker rescue has been used to establish a unique gene sequence. Marker rescue of various pairs of markers in this phage is proportional to the distance separating the pairs (8).

We have previously shown that single markers can be rescued from ϕ 105 DNA by superinfecting phage (19). The purpose of the present work is to study the relation between the genetic maps of prophage and mature ϕ 105 DNA and the position of the mutations employed on the DNA molecule. To accomplish this we have investigated marker rescue of single and double markers from prophage and mature ϕ 105 DNA. Genetic crosses were presented in a previous paper; the results are compatible with a circular map of ϕ 105 (17).

The present data, however, strongly suggest a linear map.

MATERIALS AND METHODS

Bacteria and phage. The bacterial strains employed are listed in Table 1. The *su*⁺3 suppressor was originally described by Georgopoulos (7). Wild-type $\phi 105$ has recently been described (2) as well as some of the temperature-sensitive (*ts*) mutants employed (17). The isolation of new phage mutants is described below.

Media and growth of phage and bacteria. The media employed and the general methods used for growing bacteria and phage are as described previously (17, 19).

Isolation of *ts* and *sus* mutants of $\phi 105$. For isolation of *ts* mutants, SR135 was grown in minimal CH with forced aeration at 37 C to about 5×10^7 bacteria per ml. The cells were centrifuged and resuspended in 0.5 volume of fresh medium and infected at 30 C with wild-type $\phi 105$ at a multiplicity of infection (MOI) of about 0.2. Ten minutes after infection, a 1:10 dilution was made into T broth containing 200 μ g of *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (NTG, K & K Laboratories, Plainview, N.J.) per ml. After 25 min in the presence of the drug, the bacteria were diluted 1,000-fold into T broth, and chloroform was added to the last dilution tube 85 min later. The lysate thus obtained was plated on *su*⁺ indicator at 30 C. Single plaques were picked onto two plates seeded with *su*⁺ indicator. One plate was incubated at 30 C, the other at 40 C. Phage which failed to plaque at the higher temperature was further purified and tested for its *ts* character. The mutants were given running numbers and the prefix *tsN*.

Mutagenesis to obtain suppressor-sensitive (*sus*) mutants was performed essentially as described by Tessman (23). Phage $\phi 105$ *c4*, purified by two cycles of high and low speed centrifugation, was incubated at a final concentration of about 5×10^6 plaque-forming units (PFU) per ml in 0.4 M hydroxylamine,

0.001 M ethylenediamine tetraacetic acid in 0.1 M sodium-phosphate buffer (pH 6.0) at 37 C for 18 hr. Survival of the phage was about 10^{-3} ; little killing was seen when hydroxylamine was omitted. Dilutions of the treated phage were plated with *su*⁺ indicator on plates previously seeded with *su*⁻ indicator, and the plates were incubated at 37 C. Turbid plaques were picked from these plates onto plates seeded with *su*⁺ or *su*⁻ indicator. Phage, which failed to plaque on *su*⁻, was further tested for its *sus* character. About 0.1 to 0.5% of the phages tested carried a *sus* mutation. The mutants were given a number and the prefix *sus*. Phage stocks were prepared on plates.

Phage crosses and complementation tests. Crosses were performed in *su*⁺ bacteria as previously described (17). Lysates were plated on *su*⁺ at 30 C (total phage) and at 40 C (*ts*⁺ phage), on *su*⁻ at 30 C (*su*⁺ phage) and at 40 C (*su*⁺*ts*⁺ phage). Per cent recombination was calculated as: $200 \times \text{recombinants} / \text{total phage}$. One recombination unit corresponds to 1% recombination. For isolation of *ts-sus* double mutants, *su*⁺ bacteria were mixedly infected with the appropriate pair of mutants. After burst, the progeny was plated on *su*⁺ at 30 C and plaques were picked at random on *su*⁺ at 30 and 40 C and on *su*⁻ at 30 C. Phage which failed to plaque under conditions nonpermissive for growth of either *ts* or *sus* mutants were replated, and stocks were made from a single plaque. The identity of the mutants was verified by back-crossing them with the respective parental phages.

For complementation, *su*⁻ bacteria were grown as for crosses and infected with the desired mutants at a MOI of 6 to 10 each at 40 C. After inactivation of unadsorbed phage with anti- $\phi 105$ serum, dilutions were made into T broth at 40 C, and infectious centers were assayed. Eighty minutes after infection, chloroform was added to the final dilution tubes and dilutions were plated on *su*⁺ at 30 C. Each experiment included bacteria infected with wild-type $\phi 105$ as well as bacteria infected with only one of the mutants tested.

Complementation was expressed as: $100 \times \text{burst size of mutant-infected cells} / \text{burst size of wild type-infected cells}$. We have adopted an arbitrary cut off at 10% or less as evidence of noncomplementation. In most cases noncomplementing mutants gave burst sizes of 1% or less of that of wild-type phage. The assignment of mutants to complementation groups was further checked by crossing them with the type mutant chosen to represent the particular group. We will refer to the complementation groups as genes in the following.

Preparation of DNA. Prophage DNA was extracted with phenol from the appropriate lysogenic strain as described earlier (19, 20). Mature $\phi 105$ DNA was prepared from mitomycin C-induced lysates of W168 ($\phi 105$) grown in the presence of ³H-thymidine (New England Nuclear Corp., Boston, Mass.). The phage was purified by high- and low-speed-centrifugation followed by banding in CsCl. DNA was extracted with phenol; this procedure has recently been described (20). The specific activity of ³H-labeled mature $\phi 105$ DNA was 10,000 to 20,000 counts per min per μ g.

TABLE 1. Bacterial strains

Strain	Relevant properties ^a	Origin
W 168	Prototrophic, <i>su</i> ⁻	J. Spizizen
W 168 ($\phi 105$)		J. Spizizen
SR 135	<i>SpoA-9</i> , <i>trp-7</i> , <i>su</i> ⁺ 3	J. A. Hoch
Sr 135 ($\phi 105$ <i>sus2</i>)		SR 135 lyso- genized by us
BR 95 ($\phi 105$ <i>tsN15</i>)	<i>phe-1</i> , <i>ilvA-1</i> , <i>trp-2</i>	Our collection
BR 95 ($\phi 105$ <i>tsN31</i>)	<i>phe-1</i> , <i>ilvA-1</i> , <i>trp-2</i>	Our collection

^a Properties: *su*⁺ = carries the *su* 3 suppressor gene (7), *su*⁻ = non-suppressing, *Spo* = asporogenous, *trp* = tryptophan, *phe* = phenylalanine, *ilv* = isoleucine-valine.

Shearing of mature DNA was done in 4-ml volumes in a Sorvall omnimixer with microattachment for 10 min at a rheostat setting of 90 or an average rev/min of 5,000 as determined with a Philips stroboscope type PR 9103. The container was chilled in ice during shearing. The DNA solution was 4.3 μ g per ml. Molecular weights were estimated by centrifugation in neutral sucrose gradients with radioactive lambda DNA as a marker (4). The main ϕ 105 DNA fraction had a molecular weight of 12×10^6 to 13×10^6 . Radioactivity was measured in a Nuclear-Chicago Mark I liquid scintillation counter; the techniques for measuring radioactivity have been previously described (19, 20).

Measurement of DNA synthesis in infected bacteria. *Su⁻* bacteria were grown as for crosses and infected with the appropriate phage in minimal CH at 40 C at a MOI of 6 to 10. After 10 minutes of infection, a 10-fold dilution was made into minimal CH at 40 C, and the cultures were heavily aerated. At intervals, samples were diluted one half into minimal CH containing 0.1 μ Ci of 3 H-thymidine per ml. After 1 min with the isotope, an equal volume of cold 10 per cent trichloroacetic acid was added, and the tubes were put in ice water. The samples were filtered through membrane filters (HA, Millipore Corp., Bedford, Mass.), washed three times with cold water, and dried in air; the radioactivity remaining on the filter was counted. The rate of DNA synthesis increased about twofold in the infected samples compared to uninfected bacteria between 25 to 35 min after infection and decreased at 40 to 45 min after infection, whereas the rate continued to increase in the uninfected bacteria (Fig. 1).

RESULTS

Vegetative map. To extend the previous map of ϕ 105 (17), additional *ts* mutants as well as some *sus* mutants were isolated. The relative order of the mutations was established in two-factor crosses. The mutants were further assigned to functional groups on the basis of their ability to complement each other. The results of these experiments are summarized in Table 2. Each gene is assigned a capital letter. The first number in parentheses gives the number of mutations identified in this gene, and below this is written the symbol for the mutant arbitrarily chosen to represent this gene. The numbers given above the bold-faced numbers in the Table are the recombinational values obtained in two-factor crosses. The bold-faced numbers are the per cent burst sizes of the type mutants alone under nonpermissive conditions. Below the bold-faced numbers is written per cent complementation observed between adjacent type mutants. *Sus* mutations have as yet been found in genes C, D, E, K, and L.

To verify the order of markers shown in Table 2, three-factor crosses of the type $ts_x-sus_y \times sus_z$ were performed. *Sus⁺* recombinants were selected, and the distribution of the *ts* marker among these

FIG. 1. Incorporation of 3 H-thymidine, given in 1 min pulses, into acid-insoluble material in *su⁻* bacteria at 40 C. Symbols: □, uninfected bacteria; ●, wild-type infected bacteria; ○, bacteria infected with mutant *sus7* (gene K).

recombinants was scored by testing single plaques for growth at 30 and 40 C. Of the additional mutants used in these crosses, *sus19* belongs to gene C and *tsN31* to gene K. *tsN31* is located between *sus7* and *sus11* of gene J as judged from two-factor crosses. The results of the three-factor crosses are shown in Table 3. They agree with the order of markers as derived from two-factor crosses. They give no indication of a circular map.

At this point it should be mentioned that in certain three-factor crosses of the type $ts_x - c \times ts_y$, where *c* is a clear plaque marker, *c* maps at both the right end and the left end of the vegetative map (17). However, in three-factor crosses employing *sus* markers at the right end (*sus2*, *sus7*, *sus11*) and *tsN31*, *c* maps inside both *sus2* and *sus7* (Table 4). In marker rescue, *c4* maps close to *sus11* (unpublished data). We have no satisfactory explanation for the anomalous behavior of those plaque markers in vegetative crosses.

Since gene L is located outside markers previously mapped in ϕ 105 (17), its location in the prophage was mapped by transformation in a manner previously described (16), by using donor DNA from SR135 (ϕ 105 *sus2*). The results of these crosses locate *sus2* at the prophage end closest to *ilvA-1* (Table 5). This location of *sus2* is verified by the results of marker rescue from from prophage DNA (Table 6).

Some properties of the mutants employed. None of the type mutants from any gene is significantly

TABLE 2. Vegetative map of phage $\phi 105^a$

Type mutant	Gene A (10, <i>tsN15</i>) ^b	Gene B (2, <i>tsN68</i>)	Gene C (3, <i>tsN22</i>)	Gene D (1, <i>sus13</i>)	Gene E (5, <i>tsN9</i>)	Gene F (4, <i>tsN51</i>)	Gene G (2, <i>tsN10</i>)	Gene H (5, <i>tsN34</i>)	Gene J (2, <i>sus11</i>)	Gene K (5, <i>sus7</i>)	Gene L (3, <i>sus2</i>)
<i>tsN15</i>	0.1	3.0	6.3	12.5	13.4	18.1	16.9	15.8	11.7	20.4	22.4
<i>tsN68</i>	20	0.1	1.0	6.1	6.6						
<i>tsN22</i>		43	0.2	5.8	5.2		8.0	10.4	12.0	7.0	8.0
<i>sus13</i>			26	0.1	1.8	1.0	5.0	6.1	17.0		
<i>tsN9</i>				40	0.1	1.2	4.1	4.5	10.8	21.0	15.6
<i>tsN51</i>					46	0.1	2.0	3.2		16.0	
<i>tsN10</i>						43	0.1	2.8	10.3	10.0	16.5
<i>tsN34</i>							68	0.1	6.4	7.0	9.3
<i>sus11</i>								75	0.1	2.7	7.6
<i>sus7</i>									66	0.1	3.8
<i>sus2</i>										42	0.1

^a Vegetative two-factor crosses between *ts* and *sus* mutants of phage $\phi 105$. For further details see the text.

^b Numbers in parentheses indicate, respectively, the number of mutations identified in this gene and the symbol for the mutant arbitrarily chosen to represent this gene.

TABLE 3. Three-factor crosses^a

Cross		212/012
Parent 1	Parent 2	
<i>tsN15</i> + <i>sus13</i>	× + <i>sus19</i> +	.35
+ + <i>sus13</i>	× <i>tsN15</i> <i>sus19</i> +	.10
<i>tsN15</i> + <i>sus2</i>	× + <i>sus11</i> +	.34
+ + <i>sus2</i>	× <i>tsN15</i> <i>sus11</i> +	.23
<i>tsN9</i> + <i>sus7</i>	× + <i>sus11</i> +	.40
+ + <i>sus7</i>	× <i>tsN9</i> <i>sus11</i> +	.32
<i>tsN31</i> + <i>sus19</i>	× + <i>sus13</i> +	.35
+ + <i>sus19</i>	× <i>tsN31</i> <i>sus13</i> +	.36
<i>tsN31</i> + <i>sus2</i>	× + <i>sus7</i> +	.18
+ + <i>sus2</i>	× <i>tsN31</i> <i>sus7</i> +	.11

^a The crosses were performed as described in the text. Symbols: 1 = marker from parent 1, 2 = marker from parent 2, 0 = marker from either parent. In each cross, 208 recombinants were tested for the unselected marker.

inactivated when incubated at -42°C for 90 min. Infection of *su*⁻ bacteria at -40°C leads to lysis of the cells within 2 hr except in the case of gene K mutants. In contrast to all other mutants, gene K mutants also fail to induce an increased rate of DNA synthesis after infection under nonpermissive conditions. In accordance with this finding, we have observed that after temperature-induction of a strain lysogenic for $\phi 105$ *tsN31*, where the phage also carries a mutation rendering it temperature-inducible, no infectious DNA is made under nonpermissive conditions. *Sus* mutants of gene K seem to lysogenize *su*⁻ bacteria at a strongly reduced frequency. All mutants employed in this study revert; the frequency of revertants in most stocks is 10^{-6} to 10^{-7} .

TABLE 4. Three-factor crosses with plaque marker *c4* and *ts* and *sus* mutants of phage $\phi 105^a$

Cross	Per cent clear plaques	
	<i>su</i> + At 30 C	<i>su</i> - At 40 C
+ <i>tsN31</i> + <i>x c4</i> + <i>sus2</i>	55	80
+ <i>tsN31</i> + <i>x c4</i> + <i>sus7</i>	50	80
+ <i>tsN31</i> + <i>x c4</i> + <i>sus11</i>	41	13
+ <i>tsN15</i> + <i>x c4</i> + <i>sus2</i>	59	51
+ <i>tsN15</i> + <i>x c4</i> + <i>sus12</i> (E)	57	84

^a Recombinants were plated on *su*⁻ at 40°C , and the distribution of *c4* and its wild type allele was scored among these recombinants. At least 100 recombinants were scored in each experiment.

Mapping of prophage $\phi 105$ by marker rescue. Prophage $\phi 105$ is linearly inserted into the *B. subtilis* chromosome (17). In DNA extracted from $\phi 105$ -lysogenic bacteria, the probability that two markers will occur on the same molecule depends on the size of the molecule and the distance separating the markers. If the bacterial chromosome is fragmented at random during DNA extraction, and if all markers in prophage DNA are rescued at comparable frequencies by a superinfecting phage, it should be possible to correlate the genetic map of the prophage as derived from transduction and transformation of lysogenic bacteria (16, 17) with the relative position of the mutations employed on prophage DNA measured as the relative frequency by which various combinations of markers are rescued together.

The following experiments were then per-

TABLE 5. Mapping of *sus2* in prophage $\phi 105$ by transformation^a

Recipient	Bacterial marker selected	No. of colonies analyzed	Type of prophage		
			<i>ts</i>	<i>sus</i>	++
BR95 (ϕ 105 <i>tsN15</i>)	<i>phe</i> ⁺	260	225	2	33
	<i>ilvA</i> ⁺	260	236	16	8
BR95 (ϕ 105 <i>tsN31</i>)	<i>phe</i> ⁺	260	243	3	14
	<i>ilvA</i> ⁺	260	237	14	9

^a Transformation was performed as recently described (16). Transformants were picked and tested for phage production on plates seeded with *su*⁺ (30 C total phage yielders, 40 C *ts*⁺ phage) or *su*⁻ (30 C *sus*⁺ phage, 40 C *ts*⁺ *sus*⁺ phage). After 2 hr at the respective temperature, the plates were lightly irradiated with ultraviolet to enhance plaque formation and were then further incubated overnight.

TABLE 6. Marker rescue from ϕ 105 prophage DNA^a

Superinfecting phage	Recombinational distance	Per cent co-rescue		Efficiency of rescue relative to <i>sus13</i>	
		DNA-I	DNA-II	DNA I	DNA II
<i>tsN9</i> — <i>sus13</i>	1.8	78	40	1.0	1.0
<i>tsN15</i> — <i>sus14</i>	4.4	45	26	0.6	0.9
<i>tsN15</i> — <i>sus19</i>	5.3	37		0.9	
<i>tsN9</i> — <i>sus19</i>	5.6	35	17	1.7	1.2
<i>tsN31</i> — <i>sus2</i>	6.1	23	19	0.9	1.1
<i>tsN34</i> — <i>sus11</i>	6.4		18	1.0	0.6
<i>tsN34</i> — <i>sus13</i>	8.8		10	1.0	1.0
<i>tsN15</i> — <i>sus13</i>	9.8	16	8	1.0	0.8
<i>tsN15</i> — <i>sus12</i>	12.1	8		1.0	
<i>tsN9</i> — <i>sus11</i>	13.4	6		1.0	
<i>tsN31</i> — <i>sus13</i>	14.5	4	3	1.0	1.0
<i>tsN9</i> — <i>sus7</i>	16.1	4		1.0	
<i>tsN31</i> — <i>sus19</i>	21.5	3	1	0.7	1.2
<i>tsN15</i> — <i>sus2</i>	31.5	<0.5	<0.5	0.8	0.7

^a About 0.1 μ g of wild-type prophage DNA was added to 1 ml of competent bacteria, and marker rescue was scored as described in Materials and Methods. The results give the average of two experiments. DNA I is a preparation of 30×10^6 to 40×10^6 molecular weight; it gives 3×10^5 to 6×10^5 infectious centers per ml in rescue of single *sus* markers. Background infectivity was about 0.5%. DNA II is a preparation sheared to give an average molecular weight of 15×10^6 to 20×10^6 . It gives 6×10^4 to 11×10^4 infectious centers per ml in rescue of single *sus* markers. The infectivity of this DNA was less than 10 infectious centers per microgram.

formed. Competent *su*⁺ bacteria were exposed to limiting concentrations of prophage DNA; 20 min later, the bacteria were superinfected with various *ts-sus* double mutants. The frequency of

rescue of both markers was calculated as per cent of the rescue of the *sus* marker alone. The results of these experiments are summarized in Table 6. First it is seen that all of the *sus* markers used rescue at comparable frequencies. The frequency of co-rescue of both markers was plotted as a function of the recombinational distance between the markers (Fig. 2). The frequency of co-rescue of two markers decreases in direct proportion to the distance between the markers. There is thus co-linearity between the position of the mutations on the prophage DNA and their position on the vegetative or prophage maps. Furthermore, the results indicate that the recombinational frequencies observed in two-factor crosses are a relative measure of the physical distance separating the markers.

Mapping of mature $\phi 105$ DNA by marker rescue. DNA extracted from purified $\phi 105$ particles is a collection of molecules of homogeneous size (2). We do not know whether the nucleotide sequence is identical for each molecule or whether it is randomly permuted. To distinguish between these two alternatives, marker rescue experiments were performed with whole and sheared mature DNA. Most *sus* markers rescue at comparable frequencies from whole and sheared DNA (Table 7). Marker *sus7* may rescue at a reduced frequency from at least whole DNA. All available markers in gene L rescue at a strongly reduced frequency; we will discuss marker rescue of gene L in a subsequent paper (18).

FIG. 2. Co-rescue of *sus-ts* marker pairs from prophage DNA (Table 6) as a function of recombinational distances calculated from two-factor crosses (see Table 2).

TABLE 7. Marker rescue from $\phi 105$ mature DNA^a

Superinfecting phage	Recombinational distance	Infectious centers on <i>su^c</i> at 30 C		Per cent co-rescue		Co-rescue (half:whole)
		Whole ^b	Half ^c	Whole	Half	
<i>tsN9-sus13</i>	1.8	1.0	2.1	56	45	.80
<i>tsN31-sus11</i>	1.8	2.3	4.5	32	26	.81
<i>tsN31-sus7</i>	2.6	0.4	2.4	34	14	.41
<i>tsN15-sus14</i>	4.4	1.0	1.5	31	16	.52
<i>tsN15-sus19</i>	5.3	1.9	2.9	27	16	.60
<i>tsN9-sus19</i>	5.6	2.6	2.8	22	10	.45
<i>tsN34-sus11</i>	6.4	1.5	2.4	15	14	.93
<i>tsN34-sus13</i>	8.8	2.1	1.8	12	0.8	.07
<i>tsN15-sus13</i>	9.8	1.6	3.0	18	3	.18
<i>tsN15-sus12</i>	12.1	1.1	3.6	17	1	.06
<i>tsN9-sus11</i>	13.4	1.6	1.8	8	0.7	.09
<i>tsN31-sus13</i>	14.5	2.4	4.1	12	1	.08
<i>tsN9-sus7</i>	16.1	0.6	1.7	10	1	.10
<i>tsN15-sus11</i>	25	1.6	1.6	8	0.7	.09

^a About 0.01 μ g of ³H-labeled mature $\phi 105$ DNA was added to 1 ml of competent *su⁺* bacteria, and rescue was measured as described in Materials and Methods. The results give the average of three experiments. The molecular weight of whole DNA is 24×10^6 (2). The size of the sheared (half) DNA was determined as 11×10^6 to 13×10^6 by sedimentation in neutral sucrose with either whole $\phi 105$ DNA or lambda DNA as a size marker (3).

^b Values expressed $\times 10^{-5}$.

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

In whole DNA, co-rescue of two markers decreases to about 10% with increasing distance. Co-rescue of markers separated by more than about 10 recombination units is 5 to 10% (Table 7, Fig. 3). The amount of DNA used in these experiments (0.01 μ g) is well below saturation. The level of co-rescue of distant pairs is unaffected over a 100-fold concentration range. We interpret this to mean that any two markers have about a 10% chance of being rescued independently of each other from the same molecule. Nicking of the DNA cannot explain the results; the preparation contains at the most 0.25 internal nicks per molecule (*unpublished data*).

In Fig. 4, the results of co-rescue of double markers from whole and sheared DNA are plotted as a function of the relative position of the markers on the vegetative map. Shearing also reduces the frequency of co-rescue of closely linked marker pairs; this effect seems particularly strong towards the right end of the map. However, the data suggest that the molecule possesses a shear-sensitive center between markers *tsN9* and *tsN34*, slightly to the right of the middle of the map. Thus, the data are compatible with a unique gene sequence of phage $\phi 105$.

DISCUSSION

Employing 40 *ts* and *sus* mutants, we have identified 11 essential genes in phage $\phi 105$. The molecular weight of the DNA of the phage is

FIG. 3. Co-rescue of *sus-ts* marker pairs from whole mature DNA (Table 7) as a function of recombinational distances calculated from two-factor crosses (see Table 2).

24×10^6 (2). The genome can thus harbor some 40 genes each coding for a protein containing some 200 to 300 amino acid residues. However, the whole genome of a temperate phage is not needed for growth, e.g., in coliphage lambda, 20% of the DNA may be lost without impairing growth of the phage (3, 14). The distribution of muta-

FIG. 4. Co-rescue of *sus-ts* marker pairs from half molecules/co-rescue from whole molecules plotted as a function of the relative position of the markers on the vegetative map.

tions in $\phi 105$ is not random. Of the 40 mutations analyzed, 10 are located in gene A. Therefore, it is difficult to estimate from the genetic data alone what fraction of the DNA molecule lies outside the genes identified. It seems reasonable, however, that the total map is not more than 40 to 50 recombination units long. In vegetative crosses, $\phi 105$ thus recombines with a frequency comparable to that of lambda (1), or P22 (10) and at least 1,000-fold more frequently than coliphage P2 (15).

The main object of the present experiments is to compare the genetic maps of vegetative and prophage $\phi 105$ with the frequency of co-rescue of marker pairs from wild-type mature and prophage DNA. The basic assumption of these experiments is that the frequency of co-rescue of two markers relates to the physical distance separating them. This has previously been established for SP82G (8, 9). All markers tested are rescued at comparable frequency from prophage DNA. Marker pairs are rescued together at frequencies proportional to the recombinational distance between them. As expected, the absolute frequency of co-rescue of marker pairs from prophage DNA depends on the molecular weight of the DNA preparation, but the slope of the curve (co-rescue frequency versus distance) does not change with molecular weight. These experiments thus demonstrate colinearity between the vegetative and prophage maps with respect to the position on

the DNA molecule of those mutations used to establish these maps.

It should be pointed out that the order of markers in prophage and in vegetative phage is identical. If $\phi 105$ integrates into the bacterial chromosome in the manner proposed by Campbell (6), the phage attachment site (episite) must be located outside of the presently known genes. Since at least one gene (K) essential for phage DNA synthesis has been identified, it seems that the functional organization of $\phi 105$ differs from that of coliphages lambda (21) or P2 (5).

When a collection of linear molecules is exposed to a critical shearing force, most of the molecules will break around the middle and largely half sized molecules are generated (4). By examining the frequency of co-rescue of marker pairs from whole and half molecules of mature $\phi 105$ DNA, we have sought to distinguish between a unique or permuted gene sequence. To be successful, this experiment requires that all markers are rescued at similar frequencies and that the frequency of co-rescue of two markers from the same molecule does not vary with the size of the molecule. The first criterion is not met by mature $\phi 105$ DNA since markers in gene L rescue at a strongly reduced frequency (18). Nor is the second criterion quite fulfilled since at least one closely linked marker pair (*tsN31-sus7*) shows a disproportionate decrease in co-rescue after shearing. Bearing these short-comings in mind, the results of the marker rescue experiments from mature DNA are compatible with a unique gene sequence. The first break in the molecule seems to occur preferentially between markers *tsN9* and *tsN34*. Rescue from one-fourth, one-eighth, and other size fragments should improve the results. Although single markers are rescued at measurable frequencies from one-fourth size fragments (about 1% of whole DNA), co-rescue frequencies are reduced to an extent precluding meaningful experiments. The reasons for this limitation of the experiments are unknown. Further evidence against a randomly permuted gene sequence in mature $\phi 105$ DNA will be presented in a subsequent paper (18).

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