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Genetic Map of the Staphylococcal Bacteriophage φ11

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Ten *sus* mutants of the staphylococcal bacteriophage φ11, each a representative from a different complementation group, have been used in three-factor cross experiments. The results of these crosses indicate a circular genetic map for φ11. Functional studies of the mutants have been limited to electron microscopic examinations of lysates after prophage induction (or infection). One gene is an early gene, five genes are concerned with tail formation, and three are concerned with head formation. The tenth gene is possibly a head gene. The contribution by φ11 to the genomic content of the plasmid-phage hybrid φ11*de* has been investigated. φ11*de* contains most of the late genes and appears to be missing a continuous φ11 segment that includes the early gene flanked by two late genes.

As bacteriophage are involved in many facets of staphylococcal research, we decided to initiate a genetic and biochemical study of a staphylococcal bacteriophage. We chose to study phage φ11 (4, 26) as it is active on a derivative of the *Staphylococcus aureus* strain NCTC 8325, which was the strain used in the classic genetic studies of penicillinase synthesis (28, 31). Further, phage φ11 can itself recombine with the penicillinase plasmid (26), which we felt could be a valuable property in future studies of both the phage and the plasmid.

Staphylococcal bacteriophage φ11 is a group B phage first detected as a prophage of NCTC 8325 (25, 26). Its physical and morphological properties were defined by Brown et al. (4). The phage has a molecular weight of 66.7×10^6, and contains a single molecule of double-stranded DNA of molecular weight 32.7×10^6. The intact phage particle was shown to consist of a polyhedral head, attached at one of its vertices to a flexible tail, which in turn was terminated by a complex base plate.

We previously described the isolation of 70 *sus* mutants of φ11 which were grouped into 10 complementation groups by plate complementation studies (21). In the present study a representative mutant of each group was in recombination studies. The results of these experiments indicate that φ11 has a circular genetic map. The functions of the genes represented by these mutants have been investigated, and the region of the φ11 genome absent in the phage-plasmid hybrid particle, φ11*de* (26), have been defined.

(The material in this paper represents in part the thesis submitted to the University of Adelaide by P. J. K. in partial fulfillment of the requirement for the Ph.D. degree.)

MATERIALS AND METHODS

Staphylococcal strains. The Su^- and Su_1^+ strains of NCTC 8325-4 were described (21). In that communication suppressor hosts were labeled *sup*1^+, *sup*2^+, and *sup*3^+. As this has proved confusing with the proposed genotypic symbols of Demerec et al. (9), we renamed them Su_1^+, Su_2^+, and Su_3^+, respectively, this being the phenotypic symbol commonly used (24). Strains 8325 (φ11*de*) and 8325-4 (φ11*de*) were kindly supplied by R. Novick, New York (26). NCTC 8325-4 (φ11*de*) is referred to as Su^+(φ11*de*) in this study.

Staphylococcal phages. The staphylococcal phages P47, φ11, its clear-plaque mutant φ11-M15, and the φ11 *sus* mutants were described (21). To avoid confusion with *sus* mutants, φ11-M15 has been called φ11*c* throughout this study. The nomenclature of the *sus* mutants follows that proposed by Demerec et al. (9). Suppressor sensitive (*sus*) mutants of φ11 previously described were labeled *sus*-1, *sus*-2, etc., in order of isolation, and then assigned a letter after identification of their particular complementation group. Thus, *sus*-4 became *sus*A4, *sus*-64 became *sus*E64 etc. Complementation groups were labeled with capital letters of the alphabet only after genetic recombination experiments had revealed the order and spacing (in terms of recombination percentage) of the groups on a circular map. Thus, the letters assigned indicated both gene order and gene spacing (approximately one gene per 5% recombination). It was felt that such a scheme would allow future genes to be assigned an alphabet letter without destroying order around the map, and also facilitate memory of the φ11 map. The complementation groups, which were previously assigned numbers (21), were renamed with the following letters (in parentheses); 1 (X), 2 (M), 3 (A), 4 (U), 5 (Q), 6 (H), 7 (S), 8 (O), 9 (E), 10 (P).

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Media. Unless mentioned separately, all media were as described previously (21). TC/LGC plates (used to differentiate between clear and turbid plaques) consisted of 3 ml of TC soft agar (0.5% NaCl, 1.0% tryptone [Difco], 0.7% agar [Difco], 0.004 M $CaCl_2$) overlaid on a LGC plate (1.0% tryptone [Difco], 1.0% NaCl, 0.5% yeast extract [Difco], 1.5% agar [Difco], 0.1% glucose, 0.0024 M $CaCl_2$). Ten times concentrated 0.5 CY was as for 0.5 CY, but at a 10-fold concentration. Lysing medium was equal volumes of 0.5 CY and Novick diluent. Lysostaphin buffer contained 0.05 M tris(hydroxymethyl)aminomethane, 0.145 M NaCl.

Chemicals. Erythromycin was a gift from Abbott Laboratories, Adelaide, Australia. Lysostaphin was a gift from Mead Johnson, Evansville, Ind.

General methods. Methods for growth of bacteria and preparation of phage stocks have been described (21), except that ϕ11*de*-containing strains were grown overnight in TB containing 20 μg of erythromycin per ml and grown to log phase after a 10-fold dilution in 0.5 CY containing no erythromycin (27). Optical density measurement at a wavelength of 600 nm (A_{600}) were made using a Zeiss PMQII spectrophotometer.

ϕ11 Antiserum was prepared from a rabbit immunized by a series of nine intravenous injections over a period of 3 weeks. The phage preparation containing 5 $\times$ 10^{10} PFU/ml in Novick diluent was filtered (Millipore Corp., Type HA) and 1 ml was injected. Bleeding at the end of the third week resulted in anti-ϕ11 sera with neutralization constants, K, of greater than 1,000/min (1).

Burst size experiments. One-tenth milliliter of phage (5 $\times$ 10^9 PFU/ml) was added to 0.1 ml of Novick diluent and 0.2 ml of log phase bacteria (2.5 $\times$ 10^8 colony-forming units [CFU]/ml) to give a final multiplicity of infection (MOI) of 10. After 15 min of adsorption at 37 C, ϕ11 antiserum ($K = 2$) was added. After 5 min at 37 C, the adsorption mixture was diluted 2 $\times$ 10^{-4} into lysing medium and incubated for 2 h at 37 C in a gyratory water bath shaker. Bursts of phage mutants were assayed and compared with a control run of wild-type ϕ11 or ϕ11*c*, depending on the parent from which the mutant was derived.

Liquid complementation. The method was identical to that for burst size experiments except that 0.1 ml of a second phage mutant (5 $\times$ 10^9 PFU/ml) was added to 0.1 ml of the first phage mutant and 0.2 ml of Su^- bacteria (2.5 $\times$ 10^8 CFU/ml). Control infections were performed using the one phage mutant at a MOI of 20.

Spot complementation. This method of complementation was mainly used in identification of double *sus* mutants. As such it consisted of pouring an Su^+ lawn and three Su^- lawns each seeded with a different *sus* mutant (10^5 to 10^6 per plate), two of which were the parents of the suspected double mutant, whereas the third was a complementation group different from the parental phages. The suspected double mutant (10^5 to 10^6 PFU/ml) was spotted first onto the Su_1^+ lawn and then onto the three Su^- lawns. Controls consisted of spotting each of the three phage solutions (10^5 to 10^6 PFU/ml) on all four plates. After overnight incubation (16 h) at 37 C, suspected double mutants were those which gave areas of lysis on Su_1^+ and the Su^- lawn with the third mutant, but no lysis on the Su^- lawns seeded with the parental phage.

Isolation of double sus mutants. A recombination experiment was carried out between the two parents of the proposed double mutant and progeny phage plated on Su_1^+ to give approximately 50 plaques per plate. Individual smaller plaques were transferred with a sterile toothpick into 1 ml of Novick diluent, giving approximately 5 $\times$ 10^5 to 5 $\times$ 10^6 PFU/ml, and then scanned for double mutant properties by spot complementation as indicated above.

Recombination experiments. The adsorption mixture (0.4 ml) consisted of 0.1 ml of each phage mutant (5 $\times$ 10^9 PFU/ml) and 0.2 ml of the suppressor host bacterium (2.5 $\times$ 10^8 CFU/ml). After 15 min at 37 C, the mixture was diluted 2 $\times$ 10^{-2} into lysing medium and incubated for 2 h at 37 C in a gyratory water bath shaker. The lysate was chilled, diluted, and plated on Su_1^+ to determine total progeny, and on Su^- to determine the number of wild-type recombinants. The recombination percentage is defined as double the wild-type frequency, multiplied by 100. The plating efficiency of wild-type ϕ11 on Su_1^+ compared with Su^- is 70% (P. J. Kretschmer, Ph.D. thesis, Univ. of Adelaide, Adelaide, 1974), but the recombination percentages did not include any adjustment.

Preparation of phage lysates. For UV induction of lysogens, 10 ml of a log-phase culture of the lysogen ($A_{600} = 2.5$) was resuspended in 5 ml of Novick diluent and transferred to a 9-cm-diameter glass petri dish. After 30 s of irradiation with a General Electric 15-W germicidal lamp at a distance of 50 cm (450 ergs/mm^2), 1.0 ml of 10 $\times$ 0.5 CY was added, and the solution was incubated in a 50-ml glass flask at 37 C in a gyratory water bath shaker until lysis. For lysates obtained by infection, 10 ml of log-phase cells was resuspended in 5 ml of Novick diluent and UV irradiated as above. Phage were added at a MOI of 10 (disregarding kill of cells due to irradiation) together with 1.0 ml of 10 $\times$ 0.5 CY, and incubated until lysis.

Electron microscope procedure. Phage lysates were mounted on carbon-coated grids and negatively stained with 2.0% uranyl acetate. Grids were examined using a Siemens Elmiscop I electron microscope (80 kV, 50-nm objective aperture).

Lysostaphin treatment. Lysostaphin powder was dissolved in lysostaphin buffer (200 μg/ml). An exact determination of units of enzyme activity per milliliter was not made. Lysostaphin was used only once in this study when it was added to an UV-induced culture of Su^-(*sus*A4) which had an A_{600} of 8.0. The lysostaphin (final concentration 10 μg/ml) resulted in a decrease in A_{600} from 8.0 to 0.8 in 20 min.

RESULTS

Characteristics of representative sus mutants. Each of the 10 ϕ11 complementation groups previously described (21) was represented by a single *sus* mutant throughout this study. Each representative mutant chosen was one of the first mutants isolated in the complementation group, and at that stage was the most convenient mutant of the group to use as regards leak index (defined in Table 1) and reversion rates.

The 10 representative mutants were characterized with reference to the properties shown in Table 1. As can be seen from this table, the burst size and leak index data of *sus*H47 and *sus*S71 indicate that these mutants are very leaky. However, they were successfully used in studies of liquid complementation, genetic mapping, and gene function (see below). The adsorption rates of all mutants and wild-type ϕ11 were greater than 99% in 15 min (Kretschmer, Ph.D. thesis, 1974). Mutants other than the 10 representative mutants were not as fully characterized. However, the impression gained was that mutants of the same complementation group had essentially similar characteristics.

Liquid complementation. The representative mutants of each group were subjected to liquid complementation tests, the results of which are recorded in Table 2. These tests are in accord with plate complementation results obtained previously (21). Complementation values for *sus*H47-*sus*M28 and *sus*M28-*sus*Q54 were only several-fold over the high background recorded for *sus*H47 and *sus*M28, but the mutants could confidently be placed in different complementation groups as they were separated by other complementation groups in recombination mapping.

Bursts resulting from the liquid complementation tests shown in Table 2 were also assayed on Su$^-$ to determine the frequency of wild-type recombinants. It was found that the frequency for any particular pair of mutants was equivalent to that found with Su$^+_1$ as host.

Liquid complementation experiments with mutants other than the representative mutants were carried out. Included in these experiments were the *sus* mutants A59, H61, M63, O62, U55, and X69. Mixed infection of Su$^-$ using all combinations of these and the representative mutants gave results expected from plate complementation tests and values equivalent to those of Table 2. Thus, the previous assignment of mutants into groups by plate complementation tests (21) was confirmed by all liquid complementation experiments attempted.

Recombination. Two-factor crosses. All combinations of the representative mutants were used in two-factor crosses to define a genetic map of ϕ11. However, there was considerable variation in the recombination values obtained on different days, and this variation was inconsistent in different crosses. In an attempt to stabilize the values a number of parameters were investigated (multiplicities of infection, temperature and duration of incubation, and age of culture) but a twofold variation

TABLE 1. *Characteristics of representative sus mutants of ϕ11*

ϕ11 Phage	% Burst size[a]		Leak index[b]	Plaque size (mm)	Reversion frequency[c]	Stability[d] (months)
	Su$^-$	Su$^+_1$				
ϕ11[e]	100	100		1.3		>36
*sus*A4	0.37^{3}	88^{2}	10^{9}	1.2	10^{-7}	36
*sus*E64	3.0^{3}	69^{2}	10^{4}	0.8	10^{-5}	6
*sus*H47	6.6^{6}	13^{3}	10^{1}	1.0		6
*sus*M28	2.0^{7}	7.5^{3}	10^{8}	0.8	10^{-5}	3
*sus*O43	3.0^{4}	72^{2}	10^{4}	1.2	10^{-5}	4
*sus*P68	0.30^{3}	41^{2}	10^{8}	1.0	10^{-5}	6
*sus*Q54	0.18^{4}	8.0^{3}	10^{7}	0.5	10^{-5}	1
*sus*S71	1.0^{2}	2.5^{2}	10^{1}	0.3		0.5
*sus*U53	3.3^{4}	63^{2}	10^{6}	1.0	10^{-6}	6
*sus*X27	0.19^{5}	41^{1}	10^{8}	0.8	10^{-5}	4

[a] See Materials and Methods for experimental details. Superscripts represent the number of experiments performed to obtain the average figure shown.

[b] Leak index is defined as the minimum number of PFU per plate at which leak (faint plaques on a lawn of Su$^-$) is first noticed.

[c] Reversion frequencies remain constant despite drops in phage titer. A dash indicates that the degree of leak prevented determination of reversion frequency.

[d] Average time for titer of stock to fall to 50% of original value. Stabilities of plate and liquid stocks of a particular mutant were identical.

[e] Burst size of ϕ11, which was identical to the burst size of ϕ11c, was approximately 100 PFU/cell in either Su$^+$ or Su$^-$.

persisted. Despite this variation, a preliminary map order was obtained and used as a basis for the isolation of appropriate double *sus* mutants for use in three-factor crosses. This map order was proven to be correct by the subsequent three-factor recombination experiments.

Recombination: three-factor crosses. By using the gene order indicated by two-factor cross experiments, appropriate double *sus* mutants were isolated and used in three-factor cross experiments, the results of which are shown in Table 3. In each case (but one) the observed recombination frequency was greater than the calculated value, but considerably less than the recombination frequency seen for single crossover events. The order obtained was reinforced by the fact that overlapping three-factor crosses were studied. The circular genetic map defined by the results in Table 3 is illustrated in Fig. 1. Negative interference (2, 14) was present in the ϕ11 recombination system. This can be seen from Table 3, where the interference index, i, is defined as the observed three-factor recombination percentage over the calculated percentage. The clear-plaque mutation, ϕ11c, appeared to map close to gene X.

Functional studies of representative mutants. Functional studies of the mutants involved firstly a study of the ability of mutants to cause lysis of Su$^-$ cells, and secondly, elec-

TABLE 2. *Intergroup liquid complementation of sus mutants*[a]

Complementation group	A	E	H	M	O	P	Q	S	U	X
Mutants	A4	E64	H47	M28	O43	P68	Q54	S71	U53	X27
A4	0.37^{3}	50^{2}	50^{2}	38^{2}	51^{2}	57^{1}	39^{2}	56^{2}	81^{2}	63^{1}
E64		3.0^{3}	54^{2}	29^{2}	72^{2}	50^{2}	40^{2}	56^{2}	57^{2}	43^{2}
H47			6.6^{4}	13^{3}	37^{2}	43^{2}	26^{3}	47^{2}	56^{3}	34^{2}
M28				2.0^{7}	32^{2}	31^{2}	10^{2}	38^{2}	33^{2}	30^{2}
O43					3.0^{4}	46^{2}	28^{2}	38^{2}	56^{2}	40^{2}
P68						0.3^{3}	5.0^{3}	48^{2}	77^{1}	45^{1}
Q54							0.18^{4}	22^{1}	31^{2}	20^{2}
S71								1.0^{2}	56^{2}	48^{2}
U53									3.3^{4}	42^{1}
X27										0.19^{5}

[a] Burst size measurements (each mutant at a MOI = 10) are expressed as the percentage of the burst size of wild-type ϕ11c at a MOI = 20. (In tests involving *sus*A4 the control was co-infection by ϕ11 and ϕ11c each at a MOI = 10. However there was no difference in burst size between this control and that of ϕ11c, MOI = 20). Superscripts indicate the number of experiments used to calculate the average values.

TABLE 3. *Three-factor recombination experiments*[a]

Postulated order			x	y	z Calculated	z Observed	i	x + y	No. of experiments
a	b	c							
A4	- E64	- H47	18 ± 3.0	20 ± 5.7	3.7 ± 1.4	7.5 ± 4.7	2.0	38	4
E64	- H47	- M28	22 ± 5.0	34 ± 6.8	7.4 ± 2.1	7.9 ± 4.7	1.1	56	6
H47	- M28	- O43	32 ± 11	7.6 ± 3.6	2.7 ± 1.7	3.4 ± 1.2	1.3	40	4
M28	- O43	- P68	7.0 ± 3.4	4.2 ± 1.4	0.33 ± 0.24	1.2 ± 0.53	3.6	11	5
O43	- P68	- Q54	3.9 ± 1.3	5.0 ± 2.6	0.22 ± 0.12	0.60 ± 0.18	2.7	8.9	5
P68	- Q54	- U53	5.6 ± 2.5	31 ± 6.9	1.7 ± 0.7	1.5 ± 0.38	0.88	37	3
Q54	- S71	- U53	22 ± 5.5	20 ± 7.0	4.7 ± 2.4	7.3 ± 0.56	1.6	44	3
Q54	- U53	- X27	24 ± 3.1	21 ± 3.1	5.1 ± 1.7	7.0 ± 0.90	1.4	45	4
U53	- X27	- A4	23 ± 2.0	9.5 ± 1.9	2.2 ± 0.5	4.2 ± 1.5	1.9	33	3
X27	- A4	- E64	11.0 ± 3.1	20 ± 2.1	2.3 ± 0.8	3.7 ± 2.1	1.6	31	4

[a] Recombination % = 2 × (PFU on Su$^-$/PFU on Su$^+$) × 100. x, percentage frequency of recombination observed between a and b; y, percentage frequency of recombination observed between b and c; z, percentage frequency of recombination in the cross ac × b; i, z observed per z calculated. For each experiment, a 3-factor cross and two 2-factor crosses were carried out. Due to the inherent variation of recombination frequency in the ϕ11 recombination system from experiment to experiment, average 2-factor frequencies (x or y) for any two mutants can vary from order to order.

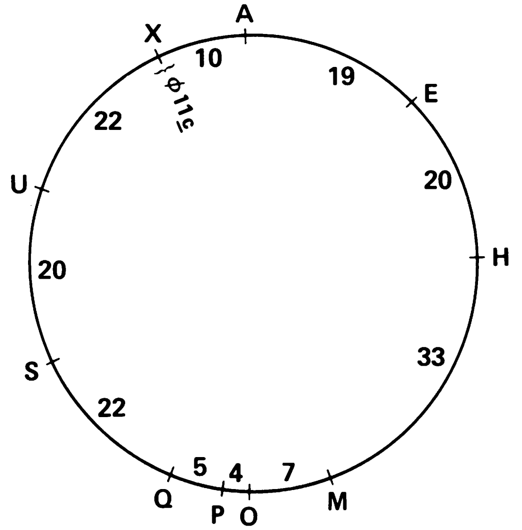

FIG. 1. *Genetic map of φ11. Figures inside the circle indicate average recombination percentage (from Table 3) between adjacent genes. The φ11c mutation maps close to gene X.*

tron microscopic examination of resulting lysates. For the latter study it was felt preferable to obtain the lysates by UV induction of Su$^-$ mutant lysogens, rather than by external infection of Su$^-$ by mutant phage which could have resulted in carry over of phage structures in the lysates. As all representative mutants except *sus*A4 were originally derived from the φ11c mutant, it was necessary first to isolate turbid-plaque representative mutants which would be capable of forming lysogens.

The *sus* mutants E64, H47, M28, O43, P68, Q54, U53, and X27 were crossed with wild-type φ11 as in a normal recombination experiment, and progeny were plated on Su^+_I, using TC/LGC medium. Such medium resulted in easy differentiation of turbid and clear plaques of φ11, or its mutants, on Su$^-$ or Su^+_I, in contrast to the poor differentiation experienced with 0.3 CY medium. After overnight incubation, smaller turbid plaques were toothpicked onto Su$^-$ and Su^+_I lawns prepoured on TC/LGC. The turbid-plaque *sus* mutants, identified as a turbid area of lysis on Su^+_I, but no lysis on Su$^-$, were further purified, and shown by spot complementation to contain the expected *sus* mutation. These turbid-plaque mutants were labeled *sus*E64c$^+$, *sus*H47c$^+$, etc. The turbid-plaque *sus* mutants E64c$^+$, H47c$^+$, M28c$^+$, O43c$^+$, P68c$^+$, Q54c$^+$, and U53c$^+$ were isolated. These had the same leak and reversion characteristics as the parental clear-plaque *sus* phage (see Table 1). No turbid plaques were isolated for the *sus*S71 and *sus*X27 mutants. The *sus*S71 plaque was faint on 0.3 CY medium, and virtually nonexistant on TC/LGC medium, and therefore plaques could not be screened. For *sus*X27, the inability to isolate the appropriate genotype probably reflected the close linkage of the *c* and X27 loci. Two thousand progeny plaques of the cross were screened, but only the *csus*$^+$ reverse recombinant was detected. Six were detected, representing a recombination frequency of 0.6% between the *c* and X27 loci. It is not known whether the absence of a *c*$^+$*sus*X27 recombinant was a chance event or not.

Su$^-$ lysogens of *sus*A4 and the above seven turbid-plaque *sus* mutants were isolated by spotting the mutants on an Su$^-$ lawn (TC/LGC medium) and subsequent purification of lysogenic cells from the center of such spots. Cultures of the mutant lysogens and of an Su$^-$ (φ11) lysogen were UV induced and incubated until lysis. For *sus*S71 and *sus*X27 the lysates were necessarily obtained by infection. To provide some validity for comparisons of the concentrations of phage structures in the lysates for the different mutants, constant initial concentrations and volumes of bacteria (lysogenic or nonlysogenic) were UV irradiated identically as indicated in Materials and Methods.

Three lysis curves are recorded in Fig. 2 and the electron microscope studies are summarized in Table 4. Except for the *sus*A4 lysogen the lysis curves for the induction of the other mutant lysogens and for the *sus*S71 and *sus*X27 infections were similar to the curve obtained for the growth of the UV-irradiated wild-type

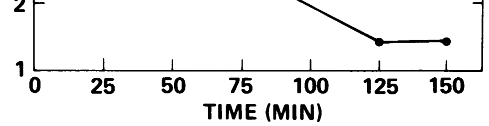

FIG. 2. *UV-induction curves of Su$^-$(φ11) and Su$^-$ (susA4). See Materials and Methods for details. Circles indicate the Su$^-$(φ11) curve, squares indicate the Su$^-$(susA4) curve, triangles indicate the control curve of UV-irradiated, nonlysogenic Su$^-$ cells. Zero time is time of addition of 10 × 0.5 CY to irradiated cells.*

TABLE 4. *Morphological observations of lysates*

Mutant lysate[a]	Tails	Heads		Whole phage		Function
		Normal	Empty	Normal	Empty	
φ11	12	24	13	20	31	
	16	14	14	25	31	
*sus*E64c^+	9	9	14	9	49	Head (?)
	11	27	18	15	30	
*sus*H47c^+	62	27	9	0	2	Head
*sus*M28c^+	57	23	24	1	0	Head
*sus*O43c^+	39	14	35	8	2	Head
*sus*P68c^+	0	27	69	1	3	Tail
*sus*Q54c^+	0	28	71	0	1	Tail
*sus*S71	12[b]	13	43	1	33[c]	Tail length
*sus*U53c^+	17[d]	11	13	37	22	Tail base-plate
*sus*X27	13	66	16	1	7	Tail

[a] All lysates were obtained by UV induction of an Su$^-$ lysogen of the respective mutant except for *sus*S71 and *sus*X27 lysates which were obtained by infection (MOI = 10) of Su$^-$ cells (see Materials and Methods). Two separate lysates of Su$^-$(φ11) and Su$^-$(*sus*E64c^+) were examined.

[b] Of the 12 tails observed in the *sus*S71 lysate ten were two to three times the length of normal tails (see Fig. 3).

[c] Of the 33 whole (empty) phage observed in the *sus*S71 lysate, three had abnormally long tails.

[d] Every tail observed in the *sus*U53c^+ lysate, whether it was free or attached to a head (i.e., a whole phage), lacked the characteristic base plate observed on wild-type φ11 phage particles (see Fig. 3).

lysogen, Su$^-$ (φ11). The UV-irradiated culture of Su$^-$ (*sus*A4) did not lyse, but followed the growth curve for the UV-irradiated nonlysogen. When an aliquot of the culture (A_{600} = 8.0) was artificially lysed with lysostaphin and examined in the electron microscope, no phage structures were seen. *sus*A4 is thus an early gene mutant.

Lysates were examined in the electron microscope after negative staining with uranyl acetate. Although dirty grids resulted from the presence of bacterial debris, lysates were not centrifuged because phage products, particularly tails, were lost. Two grids were prepared from each lysate, and 50 particles were counted and classified from random fields of view for each grid by P. Dyer of our Department. The structures were classified as tails, normal (electron dense) and empty (electron transparent) unattached heads, and normal and empty whole phage particles.

The lysate of Su$^-$ (φ11) contained approximately 15% tails, 35% heads, and 50% whole phage particles. Similar percentages of free tails and heads have been observed in electron microscopic studies of wild-type lysates of other phage systems (13, 19). The fact that some heads were empty could have reflected either the mounting and staining technique (19) or be a characteristic of phage φ11 lysates. Their presence has been observed previously for this phage (4). Their appearance at a similar frequency in a second wild-type lysate lends some significance to their appearance at higher frequency with certain mutant lysates. All lysates contained a similar concentration of total particles (heads, tails, and whole phage) except that of *sus*S71, in which the number of structures per field view was 20% that of other lysates.

Lysates of *sus*P68c^+ and *sus*Q54c^+ contained no free tails and the few whole phage particles probably reflected leak. Genes P and Q were designated tail genes. The majority of free tails in the Su$^-$ lysate of *sus*S71 infection were abnormally long, at least twice the length of the tail seen in wild-type phage lysates (Fig. 3). It therefore appeared that gene S is involved in determining the correct tail length of the phage. The lysate of *sus*X27 infection contained some tails (which could be due to a carry over of infecting phage structures), but approximately fourfold less than in the wild-type phage lysates. This fact, together with its map location at the end of the tail gene cluster distal to the head genes (see below and Fig. 4) supported its tentative assignment as a tail gene. It did differ from the other tail genes in that the heads seen in the lysates were predominantly full heads. The lysate of *sus*U53c^+ contained a similar portion of heads, tails, and whole phage as did wild-type lysates, but all tails, whether free or attached to heads, lacked the characteristic base plate described by Brown et al. (4). Figure 3 illus-

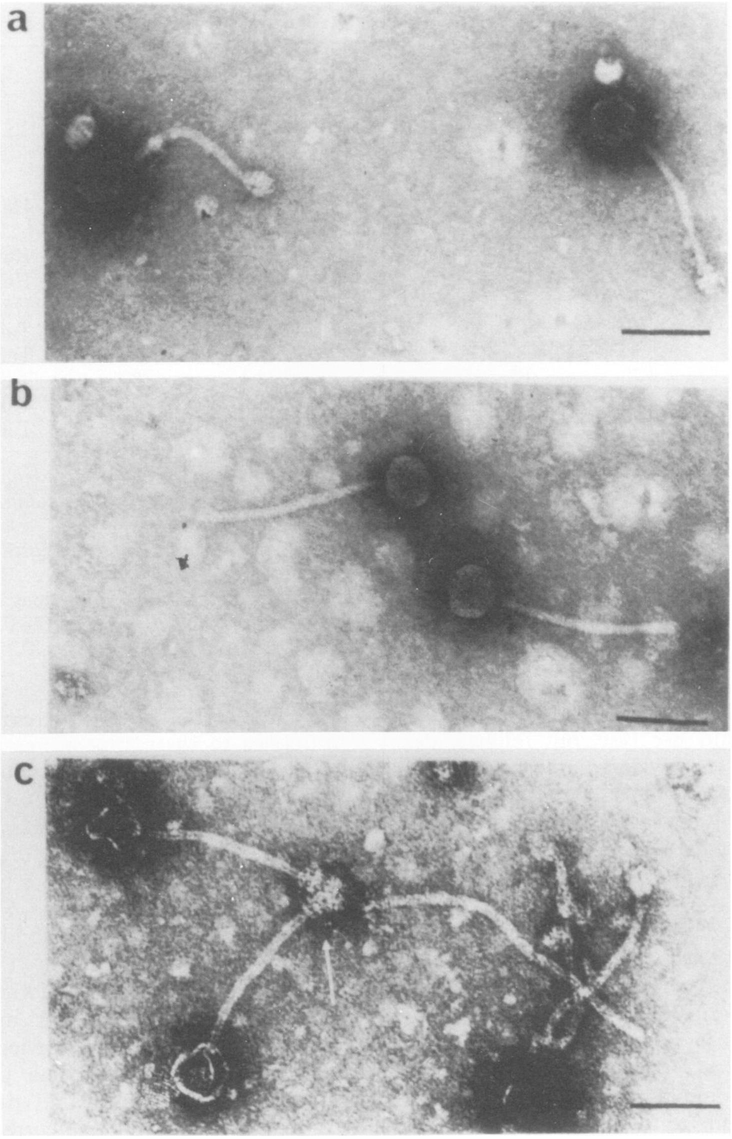

FIG. 3. *Tails observed in lysates of wild-type ϕ11, susU53c⁺ and susS71. UV induction and electron microscopic procedures were as described in Materials and Methods. (a) ϕ11 Lysate; (b) susU53c⁺ lysate; (c) susS71 lysate, the arrow indicates a clumping of tail base plates. Magnification bars = 100 nm.*

trates the difference between tails observed in the lysate of *sus*U53c^+ and those observed in wild-type lysates.

The genes H, M, and O were designated as head genes. The evidence for this was first, a significant decrease in the proportion of head structures compared with tails and second, a very low proportion of whole phage particles compared with that present in lysates of the wild-type lysogen (Table 4). Similar characteristics of head-mutant lysates have been noted in other phage systems, where such conclusions drawn from morphological studies could be verified by in vitro reconstitution experiments (19, 20, 22). The proportion of whole phage particles noted in Su$^-$ lysates of *sus*H47c^+, *sus*M28c^+, and *sus*O43c^+ could be accounted for by leak (see Table 1). In the lysate of *sus*E64c^+ the wild-type distribution of heads, tails, and whole phage was observed, but a larger proportion of whole phage particles (compared with a wild-type lysate) possessed empty heads, sug-

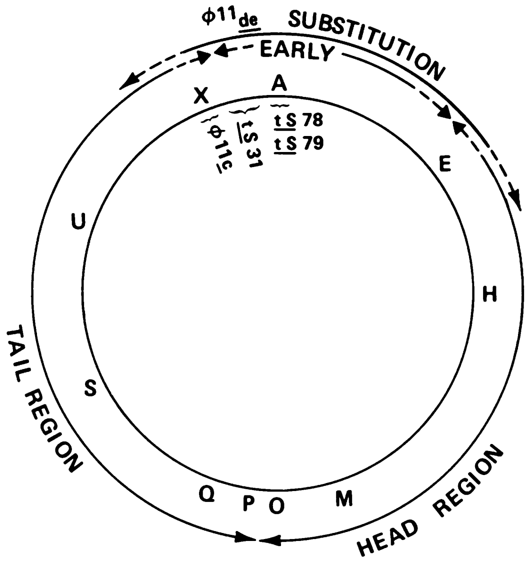

FIG. 4. *Functional genetic map of φ11. The characteristics of the mutations ts31, ts78 and ts79 were described in the text. The φ11de substitution region is that region of the φ11 genome absent from the φ11de genome, as indicated by complementation and recombination experiments (see text). The precise end points of this region in relation to the φ11 genome are unknown, as indicated by the broken lines.*

gesting an inability of the heads to incorporate the phage DNA. It is possible that the E gene is a head gene, and its map location supports this assignment of function. However, it is admitted that this evidence is by no means conclusive.

In an attempt to confirm gene assignments, lysates prepared as described in Table 4 were used in serum blocking power experiments and in vitro reconstitution studies. The technique used to assay the serum blocking power of lysates was similar to that used for P22 (16). Such experiments in the case of λ (5), T4 (12), and P22 have identified genes involved in serum blocking power as tail or tail-fiber genes. However, experiments reported elsewhere have shown that every φ11 mutant lysate afforded protection equivalent to that of a normal phage lysate (Kretschmer, Ph.D. thesis. 1974) and thus the gene(s) responsible for the production of φ11 serum blocking power were not identified.

All combinations of mutant lysates were used in attempts to demonstrate in vitro reconstitution. Equal volumes (0.3 ml) of two lysates were mixed and incubated for up to 48 h at room temperature. However, with all such combinations, and in the case of more exhaustive studies involving the lysate of the head mutant *sus*M28*c*$^+$ with lysates of the tail mutant, *sus*Q54*c*$^+$, or the base-plate mutant, *sus*U53*c*$^+$, there was no increase in phage titer (assayed on Su_1^+) above the control titers (0.6 ml of each lysate similarly treated) (Kretschmer, Ph.D. thesis, 1974).

Region of φ11 genome absent from φ11de. Complementation and recombination studies have been used to identify that region of the φ11 genome absent from the genome of the hybrid phage, φ11*de* (26). The complementation technique was essentially that of spot complementation, except that each *sus* mutant was spotted onto a lawn of 8325-4 (φ11*de*). Such complementation experiments were possible because φ11*de* lysogens do not exhibit immunity to φ11 infection (26). Such experiments indicated that φ11*de* could complement all representative *sus* mutants except *sus*A4, *sus*E64, and *sus*X27 (Table 5).

For recombination studies, φ11*de* was transduced into the Su_1^+ strain of 8325-4. A φ11*de*-containing lysate [obtained after UV induction of 8325(φ11*de*)] as described by Novick (26) was used to infect Su_1^+ (MOI = 0.3) and erythromycin-resistant transductants were selected as described by Novick and Richmond (28). An erythromycin-resistant colony was isolated and shown to be susceptible to phages P47 and φ11c, and nonlysogenic for φ11 [spotting of a log-phase culture of this colony onto a lawn of Su$^-$ gave no plaques nor area of lysis as did an Su_1^+(φ11) lysogen]. This strain was assumed

TABLE 5. *Complementation and recombination between φ11de and representative sus mutants*[a]

sus Mutant	Complementation	Recombination %[b]
A4	–	0
E64	–	0
H47	+	0.85
M28	+	1.3
043	+	4.8
P68	+	2.7
Q54	+	3.6
S71	+	0.9
U53	+	0.75
X27	–	0

[a] Complementation and recombination experiments between φ11*de* and each *sus* mutant were carried out as described in the text.

[b] Values are the average of two experiments. Values of 0 indicate no significant difference in numbers of wild-type plaques on Su$^-$ recombinant plates as compared to Su$^-$ control plates (revertant plaques). These reversion values in all cases were less than 0.01% of total phage progeny.

to be Su$_1^+$(ϕ11*de*) and used in subsequent recombination experiments.

These recombination experiments were identical to normal recombination experiments except that, as the Su$_1^+$ strain was lysogenic for one of the parental phage (ϕ11*de*), infection with only one parent (MOI = 10) was required. Su$_1^+$(ϕ11*de*) at 2.5 × 10^8 CFU/ml was infected with each representative *sus* mutant (MOI = 10). After 15 min of adsorption and a 2-h incubation period, the lysate was assayed for total progeny and *sus*$^+$ recombinants on Su$_1^+$ and Su$^-$, respectively. Controls, in which Su$_1^+$ was similarly infected with each *sus* mutant alone indicated the level of wild-type revertant plaques expected on Su$^-$ in the absence of any recombination. The results are shown in Table 5. Recombination was observed between ϕ11*de* and all mutants except the *sus* mutants A4, E64, and X27 (Table 5).

DISCUSSION

Previous studies from this laboratory have described the use of the Su$_1^+$ strain of 8325-4 in the isolation of 70 *sus* mutants of ϕ11. These mutants were shown to fall into 10 complementation groups as defined by plate complementation tests (21). The liquid complementation results reported in the present study support these plate complementation results, at least with respect to the 16 *sus* mutants tested. Leak values of the *sus* mutants are in general rather high when compared to other phage systems (6, 17, 18, 29, 32). However, these high values did not interfere with subsequent recombination experiments in which large recombination frequencies were obtained.

Three-factor crosses conclusively showed the existence of a circular genetic map for ϕ11, the overall genetic length of which is 160 recombination units. Considering the molecular weight of ϕ11 DNA as 32.7 × 10^6 (4), the recombination efficiency of ϕ11 (5 recombination units per 10^6 daltons of DNA) is 10-fold higher than that of *Escherichia coli* phage lambda (7, 8), but equivalent to that of the *Salmonella typhimurium* phage P22 (3, 30). Circular genetic maps have been demonstrated for two other generalized transducing phage, P22 (3) and ϵ^{34} (15). However, not all generalized transducing phage need necessarily have circular genetic maps, as is indicated by the demonstration of a linear genetic map for the generalized transducing phage T1 (11, 23).

Because of the lack of an in vitro reconstitution system, functional studies of the ϕ11 mutants have been limited to electron microscopic observations of mutant lysates. Gene A is an early gene, since the lysogen Su$^-$ (*sus*A4) did not lyse upon UV induction, and no structures accumulated inside the cell. Novick (personal communication) has isolated two temperature-sensitive mutants of ϕ11, *ts*-78 and *ts*-79, and found them defective in functions required early in infection. These mutants do not complement *sus*A4, and map very close to it (P. J. Kretschmer, unpublished observations).

All other genes are considered late genes since their mutant lysogens lyse after induction and phage structures are formed. Genes P, Q, and S are concerned with tail formation, and gene U with the formation of the tail base plate. Gene X has been tentatively assigned a tail function, as the frequency of tails in the lysate of the mutant infection was considerably lower than in lysates of wild-type infection. The evidence that genes H, M, and O are head genes is based on two properties of these mutant lysates in common with head-mutant lysates of other phage systems. The first is the absence of whole phage particles from the lysates (those that are observed can be accounted for by leak), and the second is the large increase in the proportion of free tail structures compared with that seen in wild-type lysates. Gene E is considered a head gene since we interpret the high frequency of whole phage with empty heads in gene E mutant lysates as reflecting the inability of the phage head to incorporate phage DNA rather than as a defect in the phage tail. The attachment of the phage tail is unnecessary for the retention of the DNA in the phage head as evidenced in the existence of full heads without tails in different mutant lysates.

When the distribution of functions around the genetic map is considered one finds a clustering of head and tail genes. The early gene A maps near the clear plaque gene *c*, which can be tentatively considered a control gene. The *c* gene most likely is situated between X and A if one is to maintain the clustering of functions as seen with other phage (3, 10, 13). Novick (personal communication) has isolated two temperature-sensitive mutants (*ts*78, *ts*79) that affect functions required early in infection and we map these next to gene A. Sjöström and Philipson (33) also map the early acting *ts*31 mutation, which affects host competence in transformation, in this region. An early region therefore can be placed between X and E (Fig. 4).

Complementation and recombination experiments indicate that the ϕ11 genes absent in the ϕ11*de* particle are contiguous, and one would expect that a continuous segment of ϕ11, from the U-X interval through the E-H interval, is missing. If the recombination map can be considered as some modification of the physical map then the missing segment is 20 to 45% of the phage chromosome. The segment deleted includes the early region. This is compatible with the facts that the *c* gene (26), the *ts*78 and *ts*79 alleles (Novick, personal communication), and the competence gene *ts*31 (33) are absent in the ϕ11*de* particle. Also missing from ϕ11*de* must be some or all of the replication genes, as the ϕ11*de* genome probably replicates as a plasmid in the bacterial cell (26). However, the initiating point of phage replication must be present on the ϕ11*de* particle, as the ϕ11*de* DNA replicates as a phage DNA when a cell harboring the ϕ11*de* particle is superinfected with ϕ11 phage (26).

In summary, we established a circular genetic map for the staphylococcal phage ϕ11 and displayed a genetic clustering of functions. We showed that in the ϕ11*de* particle the missing ϕ11 segment includes the early region together with the contiguous genes from the flanking late gene clusters.

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