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Mutations in Prophage φ11 That Impair the Transducibility of Their *Staphylococcus aureus* Lysogens for Methicillin Resistance

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Methicillin resistance (*mec*) is not transduced into *Staphylococcus aureus* 8325-4, but is transduced into this host after it has been lysogenized with phage φ11 and has acquired the penicillinase plasmid pI524 by a separate transduction (Cohen and Sweeney, 1970, 1973). Strain 8325-4 is competent for transformation of typical plasmid or chromosomal markers and for *mec* only if it is lysogenic for φ11 or a related prophage (Sjöström et al., 1974, 1975). A mutant strain of φ11 that was temperature sensitive (Ts) for vegetative multiplication did not mediate competence for transformation of its 8325-4 lysogen if the lysogen had been grown at a nonpermissive temperature (Sjöström and Philipson, 1974). We isolated four Ts mutants of φ11 that did not mediate transducibility of their 8325-4(pI524) lysogens for *mec* after growth at nonpermissive temperatures (40 to 42°C). Transduction of typical plasmid or chromosomal markers was not affected. These φ11-Ts mutants mediated normal competence of their lysogens for transformation of a tetracycline resistance plasmid. Similarly, φ11-Ts mutants that rendered their lysogens temperature sensitive for transformation did not depress the frequency of transduction of *mec*. These two types of φ11-Ts mutants fell into two different genetic complementation groups that differed in the physiology of deoxyribonucleic acid synthesis and in the time of expression of the mutations during a single-burst growth cycle at a nonpermissive temperature. A virulent mutant of φ11, which plaqued with 100% efficiency on 8325(φ11), also failed to condition strain 8325-4 for transducibility of *mec* but retained the ability to confer competence for transformation of a tetracycline resistance plasmid. Different genetic loci and physiological functions are involved in φ11 mutations that affect transducibility of *mec* and those that affect competence for transformation of markers generally in *S. aureus* 8325-4.

The temperate bacteriophage φ11, in its prophage form, has an important adjuvant effect in at least three types of genetic reactions in its native lysogenic bacterial host, *Staphylococcus aureus* 8325. When this strain of *Staphylococcus* harbors an appropriate penicillinase plasmid (usually pI524), it is transducible for methicillin resistance (*mec*), whereas its prophage-free derivative, strain 8325-4, is not. Transducibility for *mec* is regained by 8325-4 after it has acquired both pI524 by transduction and prophage φ11 by lysogenization (3, 4). The prophage requirement appears to be specific for the transduction of *mec*, since transduction of other plasmid or chromosomal genetic determinants to 8325-4 is not impaired by the absence of prophage φ11 or pI524 (4). In another type of genetic transfer, Sjöström and Philipson have shown that 8325-4 is a competent recipient in phage transfection or in the transformation of plasmid or chromosomal markers only if it harbors prophage φ11 or phage 83A as a prophage (15). In a third genetic reaction, prophage φ11 greatly enhanced the frequency of integration of thermosensitive mutant penicillinase plasmids into the chromosome of 8325-4 (14).

Sjöström and Philipson found that a mutant of φ11 that was temperature sensitive (Ts) for vegetative propagation failed to confer competence for transformation or transfection when its 8325-4 lysogen had been grown at nonpermissive temperatures (15). Following their lead, we have examined Ts mutants of φ11 for their effect on transduction of *mec* to their 8325-4 lysogens. We found that lysogens bearing mutant phages of one complementation group gave no *mec* transductants if the recipient cells had been grown at a nonpermissive temperature (42°C) before transduction. However, these cells were competent recipients for transforma-

tion of a tetracycine resistance plasmid. For ϕ11 mutants of another complementation group, the reverse was true. Lysogens bearing these mutants, grown at 42°C, were not competent recipients for transformation of a tetracycline resistance plasmid, but were effective recipients for transduction of *mec*. In addition, we have obtained virulent mutants of ϕ11 that nevertheless can lysogenize 8325-4; these lysogens are not transducible for *mec*, but still permit transformation of a tetracycline resistance plasmid. Evidently, different phage genetic functions are involved in mediation of the two types of genetic transfer.

MATERIALS AND METHODS

Bacteria, bacteriophage, and plasmids. Table 1 lists the strains of *S. aureus* and Table 2 shows the bacteriophage and plasmids employed. Mec^r and Mec^s designate the methicillin-resistant and the methicillin-susceptible phenotypes. Our methicillin resistance determinant (*mec*) was derived from *S. aureus* Villaluz, which we renamed C5 (3). For some experiments we used a mutant of this determinant, *mec-1*, with a higher degree of methicillin resistance. We obtained *mec-1* by treating a batch of phage 80 raised on C5 with ethyl methane sulfonate (0.004 M) for 30 min and then using the phage to transduce *mec* to strain 8325(pI254) with selection at 40°C. From four transductant colonies, one bearing *mec-1* was chosen for further use. For this transductant the minimal inhibitory concentration (MIC) of methicillin was ~400 μg/ml at 30°C and 15 to 25 μg/ml at 40°C, whereas strains bearing the wild-type *mec* had MICs of 50 to 100 μg/ml at 30°C and values below 5 μg/ml at 40°C.

Our strain of ϕ11 was obtained by selection of a plaque from an ultraviolet-induced lysate of strain 8325 (4). Wild-type ϕ11 and its mutants, other than virulent mutants, were carried as lysogens of strain 8325-4. Phage stocks were prepared by ultraviolet induction of the washed lysogenic cells (3). Virulent mutants were propagated by the soft-agar overlay method (1). Viable phage was enumerated by plaque formation on soft-agar overlays containing the appropriate strain of *S. aureus* after incubation at 30°C overnight (1). ϕ11*de* was a hybrid plasmid bearing genes from ϕ11 and an erythromycin resistance determinant from the penicillinase plasmid pI258. It was defective for production of plaque-forming phage (12). Four Ts mutants of ϕ11 were kindly furnished by J.-E. Sjöström. We emphasize their origin by adding the prefix SP to their designations. Suppressor-susceptible (Sus) mutants of ϕ11 and their permissive host (Su1$^+$) were the gift of P. J. Kretschmer (7, 8).

TABLE 1. *Strains of staphylococci*

Designation	Description	Source
8325	Propagating strain of international typing phage 47; Mec^s; lysogenic for prophages ϕ11, ϕ12, and ϕ13	Center for Disease Control, Atlanta
8325-4	Derived from 8325 by elimination of prophages ϕ11, ϕ12, and ϕ13	R. P. Novick (12)
C5	Mec^r strain of *S. aureus;* contains a penicillinase plasmid (pIC5) and a chromosomal streptomycin resistance determinant	(3)
Su1$^+$	Suppressor mutant of 8325-4	Kretschmer and Egan (7)
SH13e	Mutant of 8325-4 selected for resistance to phage 80	Our laboratory (4)
DU4916	Mec^r strain of *S. aureus*	(5)

TABLE 2. *Bacteriophages and plasmids*

Designation	Description	Source
ϕ11	Generalized transducing phage present as prophage in strain 8325	Isolation in our laboratory from 8325 by ultraviolet induction
ϕ11*de*	Hybrid of ϕ11 and penicillinase plasmid pI258, bearing erythromycin resistance determinant derived from the plasmid	R. P. Novick (12)
ϕ11-*ts*1, etc.	ϕ11 mutants, temperature sensitive for vegetative replication	Isolated in our laboratory
SP*ts*ϕ11$_{28}$ SP*ts*ϕ11$_{31}$ SP*ts*ϕ11$_{65}$ SP*ts*ϕ11$_{91}$	ϕ11 mutants, temperature sensitive for vegetative replication	Sjöström and Philipson (15)
ϕ11*susA4* ϕ11*susE64* ϕ11*susH47* ϕ11*susM28* ϕ11*susO43* ϕ11*susP68* ϕ11*susQ54* ϕ11*susU53* ϕ11*susX27*	Suppressor-susceptible mutants of ϕ11	Kretschmer and Egan (8)
ϕ11-*vir*1 ϕ11-*vir*2	Virulent mutants of ϕ11	Isolated in our laboratory
pI524	Penicillinase plasmid	R. P. Novick (11)
pSH1	Tetracycline resistance plasmid transduced from *S. aureus* strain 55C1	Our laboratory (17)

Media. Brain heart infusion (BHI; Difco) broth or agar, heart infusion agar (HIA; Difco), nutrient broth (NB; Difco), and Trypticase soy broth (TSB; Baltimore Biological Laboratory) were commercial products. CY broth, a casein hydrolysate-yeast broth, and phage buffer were prepared according to Novick (11).

Transduction and transformation. Transduction of *mec* was performed with phage 80 raised on *S. aureus* C5. The recipient strain was usually 8325-4(pI524), lysogenized with an experimentally varied

prophage. The need for pI524 for transduction of *mec* in this system has been reported (3). The usual concentration of methicillin for selecting Mec^r transductants, 12.5 μg/ml for penicillinase-positive recipients and 5 μg/ml for penicillinase-negative recipients, proved to be too inhibitory when used with one lot of BHI agar. This effect could be overcome by addition of 2% yeast extract.

Transformation of pSH1 was performed by the procedure of Sjöström and Philipson (15), except that the plasmid DNA was obtained by ethidium bromide-CsCl centrifugation (13, 17). Ethidium bromide was removed by extraction with isopropanol, followed by dialysis overnight against tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid buffer, pH 7.0 (17). The concentration of deoxyribonucleic acid (DNA) was estimated by absorption at 260 nm, with calf thymus DNA as a standard. Transformation was performed with a DNA concentration of 10 μg/ml, a concentration shown to be saturating.

Phage mutants. Strain 8325-4 was grown in 1% CY broth at 30°C to 5 × 10^8 colony-forming units (CFU)/ml. An inoculum of φ11, which had been subjected to three serial single-plaque passages, was added at a multiplicity of infection (MOI) of 2 to 5, and incubation was continued for 30 min. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was added to a concentration of 200 μg/ml or ethyl methane sulfonate to 0.004 M (18). After 30 min at 30°C the cultures were centrifuged, washed once with 0.85% NaCl solution, and suspended in 1% CY broth. Incubation was continued at 30°C until lysis was complete. The lysate was filter-sterilized, and dilutions were plated in overlays of HIA containing 0.5% agar and supplemented with 400 μg of $CaCl_2$/ml over base layers of HIA containing 1.0% agar. Larger plaques were produced with a bottom layer of 1% agar rather than the usual 1.5%. The Ts mutants were selected by replicating plates with well-separated plaques to 1% HIA plates previously flooded with 8325-4. Replica plates were incubated overnight at 30 and 42°C. Plaques that failed to grow at 42°C were picked from the 30°C plates, purified by three single-plaque passages, and propagated in soft overlays. Mutants that plaqued at 42°C with an efficiency of $<10^{-6}$ compared with that at 30°C were retained for further study. Lysogens were obtained by picking from discrete, turbid plaques followed by three single-colony isolations on HIA.

Virulent mutants (Vir) were obtained by picking clear plaques generated by mutagen-treated φ11 on a mixed indicator lawn of 8325-4 and 8325-4(φ11). Mutants were cloned on 8325-4. Lysogens were obtained by picking from plaques with a narrow zone of turbidity near the edge of the plaque and streaking repeatedly, until there were no more spontaneous lytic plaques. The phage obtained from these lysogens by induction with ultraviolet light produced as many plaques on a lawn of 8325-4(φ11) as on 8325-4.

Complementation tests. The standard procedure was a spot test (2). Strain 8325-4 was infected with 10^5, 10^6, and 10^7 plaque-forming units (PFU) of a φ11-Ts mutant in soft overlays of HIA, supplemented with 400 μg of $CaCl_2$/ml. Samples of 0.02-ml dilutions of another Ts mutant containing 2 × 10^4, 2 × 10^5, and 2 × 10^6 PFU were spotted on the surface of each plate. Plaques were counted after 48 h at 42°C. The presence of discrete plaques in one of the plates (usually the one inoculated with 10^6 PFU in the overlay and 2 × 10^5 PFU in the spot) was accepted as evidence of complementation. Control experiments with overlays and spots containing the same mutant or different mutants of the same complementation group produced no plaques.

Results of spot complementation tests were confirmed by tests in liquid media (2). A log-phase culture of 8325-4 in TSB at a concentration of 4 × 10^8 CFU/ml was infected with a pair of φ11-Ts mutants, each at a MOI of 5, suspended in NB with 400 μg of $CaCl_2$/ml. Serial 10-fold dilutions were plated immediately in soft-agar overlays. Plaques were counted after 48 h at 42°C. Complementing mutants usually produced 100 or more discrete plaques at a dilution of 10^{-4}. Controls gave plaques at frequencies no greater than the reversion rates of individual mutants.

Phage recombination. The procedure of Sjöström and Philipson (15) was used. With Ts mutants, strain 8325-4 at a density of 2 × 10^7 CFU/ml was infected at a MOI of 5 with each of two phages. After 10 min at 30°C in the presence of 0.004 M KCN, the mixture was diluted by a factor of 10^4 in TSB and incubated 3 h at 30°C. Recombinants were scored on lawns of 8325-4 at 42°C; total phage were scored at 30°C. Frequency of recombination was calculated according to Sjöström and Philipson (15).

Recombinants between Ts and Sus mutants were scored on 8325-4 at 42°C; total phage were scored on Sul$^+$ at 30°C.

Phage DNA synthesis. The procedure of Studier (18) was followed. Strain 8325-4, grown at 40°C in CY broth with added 400 μg of $CaCl_2$/ml to early-exponential phase (10^8 to 2 × 10^8 CFU/ml), was infected with phage at a MOI of 5. Incubation was continued at 40°C. Samples of 1 ml were withdrawn at intervals of 15 min and placed in tubes at 40°C containing 10 μl of [^{3}H]thymidine (0.1 μCi/ml; 43.9 Ci/mmol) in 0.1 ml of water and held for 1 min at 40°C. Cold 10% trichloroacetic acid, 1.2 ml, containing 100 μg of thymidine/ml was then added. After 30 min at 0°C, the precipitate was collected on glass-fiber filters (Whatman GF/C), washed three times with cold 0.01 N HCl and once with 5 ml of cold 95% ethyl alcohol, dried, and counted in a scintillation spectrometer. In these experiments, zero time corresponds to the addition of phage.

Temperature-shift experiments and phage growth. Strain 8325-4 was grown to early-exponential phase (2.5 × 10^8 cocci/ml) in CY broth at 30°C. The cells were collected by centrifugation, suspended in phage buffer at a concentration of 2 × 10^8/ml, and infected with an equal volume of phage suspension in phage buffer at a MOI of 5. After incubation at 30°C for 10 min, the cells were collected by centrifugation, suspended in CY broth at 2 × 10^8/ml, and incubated at 30°C. Aliquots were withdrawn at varying times, incubated at 40°C for 120 min, and plated for infectious centers. In these

experiments, zero time corresponds to the time of dilution of the phage-staphylococci mixture into CY broth.

RESULTS

ϕ11-Ts mutants and *mec* transduction. We lysogenized strain 8325-4(pI524) with a plaque-purified clone of each of 54 Ts mutants of ϕ11. Each lysogen was grown overnight in TSB separately at 30 and 42°C. The cocci were collected by centrifugation and tested for ability to receive *mec* by transduction at the standard temperature, 37°C. All lysogens grown at 30°C were effective recipients, but four phage mutants (*ts*2, *ts*4, *ts*9, and *ts*26) gave lysogens that were not transducible for *mec* after growth at 40 to 42°C (Table 3). Lysogens made with 15 thermally insensitive revertants from three of these mutants were in each case effective recipients for *mec* after growth at 42°C. Lysogens made with the other Ts mutants were effective transductional recipients of *mec* after growth at 42°C, but the frequency of transduction was somewhat reduced over that with the same cells grown at 30°C, as was the case with wild-type ϕ11. We obtained similar results with the transducibility of *mec* from another Mec^r strain, DU4916 (data not shown). Therefore, the conditional non-transducibility of lysogens of the four exceptional Ts mutants is not a peculiarity of the C5 *mec* determinant.

The kinetics of loss and regain of recipient effectiveness with growth at nonpermissive temperatures are depicted in Fig. 1. In these experiments, exponential-phase cultures of 8325-4(ϕ11-*ts*4)(pI524) growing at 42 or 30°C in BHI broth were diluted with fresh broth at the reciprocal temperature to a cell concentration of 1.3×10^8/ml. Exponential-phase growth was continued at the new temperature with vigorous orbital shaking by means of twofold dilutions in fresh broth when the cell density doubled (measured by absorbance at 540 nm). Samples were withdrawn at the indicated times and the cells were collected by centrifugation, resuspended in NB with $CaCl_2$ supplement, and used for transduction of *mec*, with selection at the standard temperature, 30°C. The results, generally similar to those obtained by Sjöström and Philipson (15) in comparable experiments on transformation of genetic markers, showed that from three to four generations of growth were needed to reverse the effect of prior growth at either 30 or 42°C. Incubation of a *ts*4 lysogen in phage buffer solution for periods of time up to 5 h, at either of the foregoing temperatures, did not change the transducibility for *mec* determined by the prior growth at 30 or 42°C. Similarly, incubation in BHI broth containing 50 μg of chloramphenicol/ml or 0.004 M KCN did not alter transducibility. Thus, the temperature-related inhibition or restoration of transducibility for *mec* of strain 8325-4 lysogenized by these four ϕ11 mutants was dependent

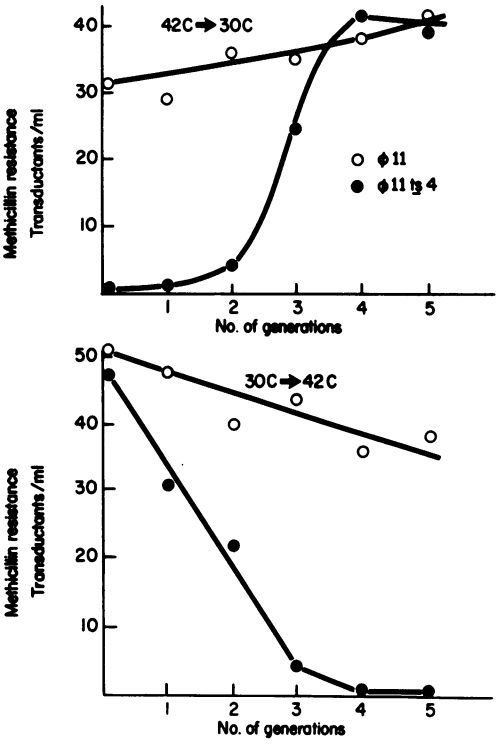

FIG. 1. *Effect of a shift in temperature of growth on transducibility for mec of ϕ11 lysogens of 8325-4(pI524). At zero time the temperature of growth of the recipient culture was shifted as indicated in the figure. Number of generations was determined by absorbance at 540 nm. Symbols:* (●) *mec transductants with 8325-4(ϕ11-ts4)pI524),* (○) *mec transductants with 8325-4(ϕ11)(pI524).*

TABLE 3. *Frequency of transduction of mec to lysogens of phage ϕ11-Ts mutants*

Designation of mutant[a]	Frequency of transduction of *mec* in recipients grown at:	
	30°C	42°C
*ts*2	3.3×10^{-8}	$<10^{-9}$[b]
*ts*4	5.2×10^{-8}	$<10^{-9}$[b]
*ts*30	4.3×10^{-8}	2.5×10^{-8}
*ts*6	9.2×10^{-8}	7.8×10^{-8}
*ts*11	9.8×10^{-8}	4.5×10^{-8}
Wild type	1.0×10^{-7}	7.1×10^{-8}

[a] Results are given for representative phage mutants.

[b] No *mec* transductants were obtained with lysogens of these mutants grown at 42°C.

on a process that was closely related to growth of the lysogens.

Genetic experiments with Ts mutants. Complementation tests, performed at 42°C, by all possible paired combinations of the 54 independent Ts mutants of ϕ11 indicated that they comprised three complementation groups (Table 4). The four mutants that rendered their 8325-4 lysogens nontransducible for *mec* after growth at 40 to 42°C were all in group 1. For convenience of reference, we have designated these four mutants as group 1b. The other five group 1 mutants, designated group 1a, made normally transducible lysogens. The presence of three complementation groups among the ϕ11-Ts mutants was confirmed by recombination experiments. No temperature-insensitive recombinant phage was detected in crosses between mutants of the same group, including crosses between group 1a and 1b mutants, whereas intergroup crosses gave recombination rates of 0.07 to 0.68%. Thermally insensitive progeny of intergroup crosses involving Ts group 1b mutants mediated transducibility of *mec* in their 8325-4 lysogens after growth at 42°C (39 progeny strains from eight crosses).

Complementation tests with our Ts mutants and four ϕ11-Ts mutants kindly supplied by Sjöström (SPts_{28}, SPts_{31}, SPts_{65}, and SPts_{91}) showed that our group 2 mutants gave no complemetation with the ts_{31} mutant of Sjöström and Philipson. Representatives of our groups 1a, 1b, and 3 mutants complemented each of their other mutants and therefore reflect mutations at different sites.

Recombination experiments were performed between two ϕ11 mutants from each of our complementation groups and six Sus mutants of ϕ11 (Table 5). The results with our group 2 mutants closely resembled those of similar crosses by Sjöström and Philipson between Sus mutants and their ts_{31} mutant (15), confirming the probable identity of our group 2 and their group II, exemplified by ts_{31}. On the other hand, results of crosses between Sus mutants and our group 1a, 1b, and 3 mutants deviated significantly from those with the Sus mutants and the Ts groups I, III, and IV mutants of Sjöström and Philipson and did not lend themselves to reliable location in the map of the ϕ11 genome of Kretschmer and Egan (8). We have no plausible explanation for these latter results.

TABLE 4. *Complementation groups among ϕ11-Ts mutants and transducibility of mec*

Complementation group	No. of ϕ11 mutants tested for transducibility of *mec* to their lysogens in strain 8325-4(pI524) after growth at 42°C[a]	
	Effective recipients	Ineffective recipients
1	5	4
2	18	0
3	27	0

[a] Results at 40°C were virtually the same as those at 42°C.

TABLE 5. *Recombination between Sus and Ts mutants of ϕ11*[a]

sus mutant	Recombination rate with Sus mutant in ϕ11 group		
	1	2	3
A4	0.43	0.0108	1.125
O43	1.07	1.14	0.55
P68	2.9	7.0	0.27
Q54	0.587	4.48	0.148
U53	3.65	1.12	0.064
X27	0.75	0.107	0.012

[a] Results are means of single experiments with each of two mutants from each complementation group.

Complementation tests at 40°C with our Ts mutants and ϕ11*de* disclosed that ϕ11*de* was complemented by our group 1a, 1b, and 3 mutants, but not by our group 2 mutants. This result was in accord with the observation by Sjöström and Philipson (15) that ϕ11*de* was not complemented by their group II mutants. Complementation with 1b mutants suggested that ϕ11*de* should be able to substitute for ϕ11 in conditioning 8325-4(pI524) for transducibility of *mec* after growth at 42°C. Such an experiment could not be performed with our standard recipient strain, since ϕ11*de* retains the incompatibility determinant of its parental plasmid and therefore cannot coexist stably with pI524. To circumvent this problem we employed as a recipient strain SH13e, a variant strain of 8325-4 selected for resistance to a high concentration of phage 80 (4). Even though it did not contain a penicillinase plasmid or obvious phage genes, strain SH13e was weakly transducible for *mec*, as were some other strains selected in the same way. The mechanism of this change in transducibility is unknown. The effectiveness of SH13e as a transductional recipient for *mec* after growth at 42°C was enchanced by prior lysogenization with wild-type ϕ11 but not by group 1b mutants (Table 6). Although the increment in frequency of transduction was not very great, it was reproducible. In this host, ϕ11*de* had an effect comparable to that of ϕ11, and their combined effect in the same cell was approximately additive, a result that suggests a gene dosage effect.

TABLE 6. *Enhancement of transduction of mec by φ11de*

Recipient	No. of transductants[a]
SH13e	6
SH13e(PI258)	5
SH13e(φ11)	39
SH13e(φ11*de*)	28
SH13e(φ11)(φ11*de*)	64
SH13e(φ11*ts*4)	4[b]
SH13e(φ11*ts*4)(φ11*de*)	31[b]

[a] Expressed as transductants per milliliter of phage (2.1×10^9 PFU/ml).

[b] Tested after growth of these lysogens at 42°C. Other recipients were tested after growth at 30°C.

The temperature-dependent inhibition of transduction of *mec* in the 1b group of φ11 lysogens might have been caused by a failure of expression of *mec* in the recipient at the nonpermissive temperature. To investigate this question we used the mutant determinant, *mec-1*, with an increased degree of methicillin resistance. The native temperature sensitivity of expression of the wild-type *mec* determinant in strain C5 makes quantitative assessment of the degree of methicillin resistance mediated by it unfeasible at 40 or 42°C. Whereas transduction of *mec-1* into 1b φ11 lysogens of 8325-4 was inhibited by growth of the lysogens at 42°C, the expression at this temperature of an established *mec-1* was not different from that in lysogens bearing wild-type φ11 or other Ts mutants (Table 7).

Physiological observations on φ11-Ts mutants. The effect of incubation at 40°C upon phage DNA synthesis in strain 8325-4 infected with φ11 and its Ts mutants is indicated in Fig. 2. The data indicate that phage DNA was synthesized at nearly normal rates in cells infected by representatives of group 1 or 3 complementation groups of Ts mutants. Infection by a group 2 mutant was followed by virtually no incorporation of the thymidine label either into phage or host cell DNA. Similar results were obtained with another set of representatives of each of the complementation groups of Ts mutants.

The foregoing results suggested that the group 2 mutants involved a relatively early function in the phage biosynthetic sequence, and the group 1 mutants involved a later function. This inference was confirmed by temperature-shift experiments (Fig. 3). These show that φ11-*ts*6 (group 2) and φ11-*ts*14 (group 3), required 20 to 30 min of vegetative growth at 30°C to pass the points of the physiological blocks imposed by a shift to the nonpermissive temperatures. For group 1a or 1b mutants, the corresponding time was 30 to 40 min.

These data confirm the evidence from the genetic eperiments that group 1 and group 2 mutations in φ11 affect distinctly different functions. In other experiments, no major difference was noted among the three complementation groups. Cells of 8325-4 infected with a mutant from each group grew normally at 40°C in TSB. Lysis of these cells by treatment with lysostaphin released no phage that formed plaques at 30°C, although we were able to show that similar premature lysis of cells infected with wild-type φ11 did release appreciable numbers of phage.

Transformation of pSH1. To compare the effect of the Ts mutants of φ11 upon transfor-

TABLE 7. *MIC of methicillin at 42°C for S. aureus 8325-4(pI524)mec-1 bearing Ts mutations of prophage φ11*[a]

Complementation group of φ11 mutant	MIC (μg/ml)	
	50%	100%
Wild type	18	35
1a	15	25
1b	16	30
2	21	60
3	16	49

[a] *mec-1* was transduced at 30°C into lysogens bearing the different φ11-Ts mutants. The transductants were grown overnight at 40°C, suitably diluted, and aliquots were plated on BHI medium containing serial twofold dilutions of methicillin. The number of colonies apparent after 2 days at 42°C was enumerated. The 50% MIC designates the concentration of methicillin that reduced colonies formed to 50% of the control value. The 100% MIC eliminated colony formation completely. Results are geometric means of two to four determinations with one or two independent lysogens.

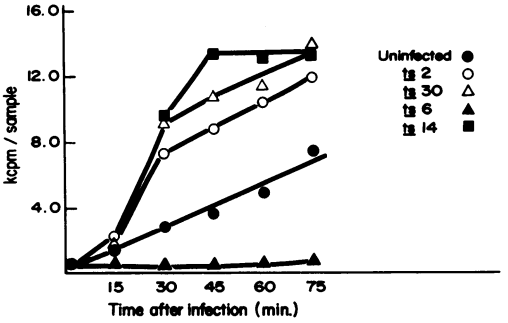

FIG. 2. *DNA synthesis determined by pulse labeling with [³H]thymidine during vegetative propagation of φ11-Ts mutants on strain 8325-4 at 40°C. For procedure, see Materials and Methods. Symbols:* (○) *φ11-ts2 (group 1b),* (△) *φ11-ts30 (group 1a),* (▲) *φ11-ts6 (group 2),* (■) *φ11-ts14 (group 3). Results with wild-type φ11 (data not shown) resembled those with φ11-ts30.*

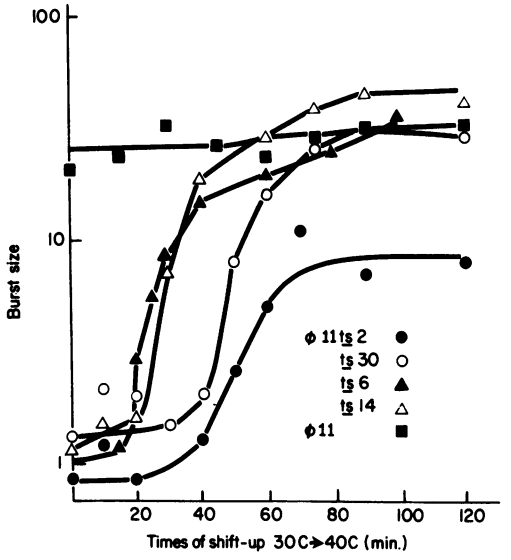

FIG. 3. *Effect of temperature shift at varying times after infection on the burst size of φ11-Ts mutants. For procedure, see Materials and Methods. Symbols: (●) φ11-ts2 (group 1b), (○) φ11-ts30 (group 1a), (▲) φ11-ts6 (group 2), (△) φ11-ts14 (group 3), (■) φ11 (wild type).*

mation of markers into 8325-4, we used the DNA of the tetracycline resistance plasmid pSH1. Table 8 shows that competence of 8325-4, bearing group 1a, 1b, or 3 Ts mutants of φ11 grown at 40°C, was unimpaired, whereas strains bearing group 2 mutants were almost completely noncompetent. It should be noted that pSH1 could be transduced into lysogens of group 2 mutants with normal frequency, irrespective of the temperature of growth of the recipient. These results were in accord with the assumption that our group 2 mutants were equivalent to the group II mutants of Sjöström and Philipson, which failed to mediate competence for transformation of φ11*de* when their lysogens were grown at nonpermissive temperatures.

Other φ11 mutants and transducibility of *mec*. One other class of φ11 mutants also produced lysogens that were selectively nontransducible for *mec*. Lysogens of φ11-*vir*1 (See Materials and Methods) in 8325-4(pI524) grown at 30 or 42°C gave no Mec^r transductants, whereas they were competent for the transformation of pSH1 at normal frequencies. Transduction of pI524, pSH1, or chromosomal streptomycin resistance took place in these strains with normal frequencies. Revertants of φ11-*vir*1 detected by their formation of normally turbid plaques on 8325-4, made lysogens that were normally transducible for *mec*. Results with lysogens of φ11-*vir*2 were similar.

These preliminary observations indicate that a mutation affecting the regulation of vegetative phage multiplication also altered the transducibility for *mec* of lysogens bearing these mutant phages without detectably affecting transformation of a plasmid.

We were able to establish lysogens of seven φ11 Sus mutants in 8325-4(pI524). In each case ultraviolet induction of the lysogens produced a crop of phage that titrated at 10^4 to 3×10^5 PFU/ml on Su1$^+$ and 0 to 250 PFU/ml on 8325-4. Each of the Sus lysogens was transducible for *mec* after growth at 30°C (Table 9). Lysogens of two φ11 Sus mutants, M28 and U53, were exceptional in having a very low level of transducibility after growth at 42°C. Overall, however, it does not appear that the Sus mutants tested were notably defective in mediating transducibility for *mec*.

DISCUSSION

Our results confirm that one or more functions of phage φ11 in the prophage state are essential for the transduction of *mec* to *S. aureus* 8325-4. Mutations at either of two sites, not obviously functionally related to each other, eliminated this permissive effect of prophage φ11. The fortuitous detection of an effect on transducibility of *mec* in two of the relatively few types of phage mutations examined thus far raises the possibility that still other phage mutations may affect *mec* transduction. The class 1b φ11-Ts mutants, which inhibited the transducibility of *mec*, did not differ from class 1a mutants, which do not inhibit this function, in any of the genetic or physiological tests applied to date. The 1b mutants may contain

TABLE 8. *Prophage φ11-Ts mutants and transformation of a tetracycline resistance plasmid*

Prophage φ11 mutants		Transformation frequency of tetracycline resistance (no./viable recipient) in recipients grown at:	
Complementation group	Designation of mutant	30°C	40°C
1a	*ts*30	8.1×10^{-8}	4.9×10^{-8}
1a	*ts*46	1.2×10^{-7}	4.1×10^{-8}
1b	*ts*2	3.4×10^{-8}	1.3×10^{-8}
1b	*ts*4	2.7×10^{-8}	1.1×10^{-8}
2	*ts*15	1.5×10^{-7}	$<10^{-9}$ [a]
2	*ts*21	1.6×10^{-7}	$<10^{-9}$ [a]
2	*ts*38	NT[b]	1.7×10^{-9}
3	*ts*14	1.4×10^{-7}	5.5×10^{-8}
3	*ts*16	1.6×10^{-7}	7.0×10^{-8}

[a] No transformants were obtained.
[b] NT, Not tested.

TABLE 9. *Transducibility for mec of lysogens of φ11 Sus mutants in 8325-4*

φ11 Sus mutant used for lysogenization	No. of *mec* transductants with staphylococcal host strain			
	Su1⁺(pI524)		8325-4(pI524)	
	30°C	42°C	30°C	42°C
M28	58	32	73	3
H47	46	28	89	47
U53	32	18	44	2
Q54	33	19	25	14
P68	28	12	21	12
E64	49	28	71	29
O43	36	15	20	13
Wild type	81	38	68	36

[a] Expressed as transductants per milliliter of phage (1.4×10^9 PFU/ml).

additional undetected mutations responsible for their effect on *mec* transduction, but this seems unlikely since their spontaneous temperature-insensitive revertants and recombinants regained ability to mediate transducibility of *mec*. The group 1a and 1b mutants may bear lesions in the same gene but may differ in the site or mechanism of mutation, thereby defining gene products with different effects on transducibility of *mec*. Alternatively, 1b mutants may have polar effects on adjacent genes that are unaffected by the 1a mutations.

Although we were stimulated to examine φ11-Ts mutants for effects on *mec* transduction by the earlier reports of their effect on transformation of *S. aureus* (15), it is apparent that, in these mutants, the genetic locus affecting transduction of *mec* differs from that related to transformation of a tetracycline resistance plasmid or the hybrid plasmid φ11*de*. Furthermore, the fact that either *mec* or a tetracycline resistance plasmid could be transduced into 8325-4 bearing a class 2 mutant prophage φ11 indicates that this mutation, which inhibited transformation of these markers, did not affect mechanisms fundamental to their expression, establishment, or maintenance. This observation is in accord with the evidence of Sjöström and Philipson that φ11 lysogeny facilitates transformation in 8325-4 through an effect on the competence of the recipient cell (15). To complete the comparison, it will be desirable in future work to determine whether *mec* may be transformed into 8325-4 lysogens that bear 1b φ11 mutant prophages and have been grown at nonpermissive temperatures.

The step in the transduction of *mec* at which prophage φ11 acts remains obscure. We presume that transducing DNA is injected normally into strains of 8325 that are nontransducible for *mec*, since transduction of markers other than *mec* proceeds with normal frequency. The *mec* DNA may be subject to restriction in the nontransducible strains, but if that is the case, the restriction mechanism must be highly specific for *mec* in view of the normal transducibility of other markers. Failure of expression of the transduced *mec* in lysogens of 8325-4 bearing 1b φ11 mutants seems an inadequate hypothesis, since we have shown that an established *mec* gene is normally expressed at a nonpermissive temperature in 8325-4 lysogenic for 1b φ11 mutants. Therefore, we assume that the defective function in the 1b φ11 lysogens is related to establishment and maintenance of *mec* in its host. It seems likely that this process, mediated by the combined presence of prophage φ11 and pI524, involves recombination of *mec*, presumably with the staphylococcal chromosome, a hypothesis that is supported by the absence of detectable extrachromosomal DNA attributable to *mec* (9, 16, 17) and the increase in rate of transduction of *mec* after ultraviolet irradiation of transducing phage (3). Strong evidence for a chromosomal locus for *mec* was adduced by Sjöström et al. in experiments demonstrating the transformation of *mec* by chromosomal fractions of donor DNA (16). Like the transduction of *mec*, its transformation was dependent on φ11 lysogeny of the recipient 8325-4. However, the transformation did not require the presence of a penicillinase plasmid in 8325-4(φ11), was not enhanced by ultraviolet irradiation of the transforming DNA, and occurred with equal frequency in a mutant of 8325-4 with *rec*A⁻ properties. Sjöström et al. suggested that their results might be explained by the hypothesis that *mec* was located on a transposition sequence, which could be inserted into the chromosome without benefit of conventional recombination mechanisms (16). We have confirmed that the transduction of *mec* from DU4916, the strain used by Sjöström et al. behaves like our strain C5 *mec* determinant with respect to the penicillinase plasmid requirement, ultraviolet irradiation of transducing phage, and effect of our φ11 Ts mutants (S. Cohen and H. M. Sweeney, unpublished data). Furthermore, in preliminary experiments, we have found that *mec* was transduced into the same *recA*⁻ mutant of strain 8325-4 (bearing φ11 and pI524) as that employed by Sjöström et al., albeit at frequencies relative to those obtained with 8325-4 that were appreciably less than those for pSH1 or a chloramphenicol resistance plasmid. A typically chromosomal determinant, i.e., streptomycin resistance, gave no transductants with this *recA*⁻ recipient. Therefore, it appears that *mec*

DNA is handled differently within the recipient cell, depending upon whether it was introduced by transduction or transformation. We suggest that the differences in the factors affecting genetic transfer of *mec* by these two methods may be related to the processing of DNA in transformation and transduction. In transformation, donor duplex DNA is separated, at least in part, into single strands, followed by insertion of a single strand of DNA into the recipient chromosome (10). In transduction, a shorter segment of double-stranded DNA is injected, which may be used directly for recombination (6). Our results indicate that the transfer of *mec* by these mechanisms calls upon different types of adjuvant support by prophage ϕ11, pI524, ultraviolet irradiation and, tentatively, *recA* function.

The unusual properties of *mec* with respect to transformation and transduction, together with its ability to be eliminated from some host strains, either spontaneously or secondary to elimination of prophage ϕ11 (4, 9), suggest that *mec* is not an ordinary plasmid or chromosomal genetic determinant and lend credence to the suggestion of Sjöström et al. (16) that it is situated on a transposition sequence.

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