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# Distribution of Tn*551* Insertion Sites Responsible for Auxotrophy on the *Staphylococcus aureus* Chromosome

PETER A. PATTEE

*Departments of Microbiology and Molecular, Cellular, and Developmental Biology, Iowa State University, Ames, Iowa 50011*

A method was devised to efficiently select isolates of *Staphylococcus aureus* 8325 in which Tn*551*, a transposon originating on the pI258 plasmid and responsible for erythromycin resistance (Em$^r$), had translocated to the host chromosome. This method consisted of selecting for Em$^r$ at 43°C with a strain in which the pI258 plasmid was unable to replicate at 43°C because of a temperature-sensitive plasmid mutation. By selecting isolates that were Em$^r$ at 43°C and auxotrophic for nutrients not required by the parent strain, Tn*551*-induced auxotrophic mutants were readily isolated. The incidence of auxotrophic classes was not random; 80% of the isolates in one experiment were Trp$^-$, whereas only a single example of each of some of the other classes was isolated. Among the Trp$^-$ mutants, the distribution of *trp* genes affected and the frequency of precise excision of Tn*551* from individual sites varied. When analyzed by transformation, the Tn*551*-induced *ala, his, ilv, lys, rib, thrA, thrB,* and *trp* mutations were shown to occupy sites previously defined by nitrosoguanidine-induced mutations. Tn*551*-induced mutagenesis provided three previously unrecognized classes of auxotrophs (*tyr, met,* and *thrC*), and the Tn*551* integration sites resulting in these mutations have been identified. In addition, a chromosomal region (*uraB*) was identified by Tn*551* mutagenesis that is distinct from *uraA* (previously defined by chemical mutagenesis). Some Tn*551*-induced mutations (most notably *pur*) could not be linked to the known linkage groups of the chromosome by transformation. With the exception of two *pur* mutations, all of the Tn*551*-induced auxotrophic mutational sites cotransformed at unity with Tn*551* and, in cases in which they were selected, prototrophic transformants were always Em$^s$. Thus, the Tn*551* and auxotrophic sites are identical.

In *Staphylococcus aureus,* transformation has defined three clusters of genetic loci that are cotransformable (17). Subsequent additions to these linkage groups (1, 10, 14, 16), particularly by mapping a limited number of sites occupied by the erythromycin resistance (Em$^r$) transposon Tn*551* (13, 18), have raised the possibility that transformation offers a means of defining the chromosome of this bacterium. Figure 1 summarizes the status of the three known linkage groups in *S. aureus* 8325 at the onset of this study.

There were two problems associated with the further exploitation of these Tn*551* insertion sites for purposes of mapping the chromosome. First, the Tn*551* insertions used in these studies were relatively rare and involved the transposition of Tn*551* from the pI258 plasmid to the recipient chromosome during transduction. They were isolated by Novick (11) who used UV-treated lysates prepared on pI258-bearing donor strains to transduce Em$^s$ recipients; the linkage of the plasmid-borne markers (β-lactamase-mediated resistance to penicillin [Pc$^r$], Em$^r$ [due to *ermB* on Tn*551*], and several determinants of resistance to heavy metals) during transduction usually was preserved at high efficiency. Only rarely were Em$^r$ transductants recovered that lacked the other plasmid-borne markers, and it was this rare class of transductants that proved to possess Tn*551* at various chromosomal sites (13, 18). Second, these Tn*551* insertions were defined only by the Em$^r$ phenotype; i.e., none of them influenced the functions of chromosomal genes in any phenotypically apparent way. As a consequence, whereas three known extremities of the linkage groups were extended significantly by Tn*551* sites (Ω11, Ω34, and Ω40; Fig. 1), these markers could not be tested for linkage to one another because they all conferred only the Em$^r$ phenotype.

The isolation of thermosensitive mutants of pI258 by Novick (12) provided the means of overcoming these difficulties. When a strain carrying a thermosensitive plasmid is cultivated in the presence of erythromycin at the nonper-

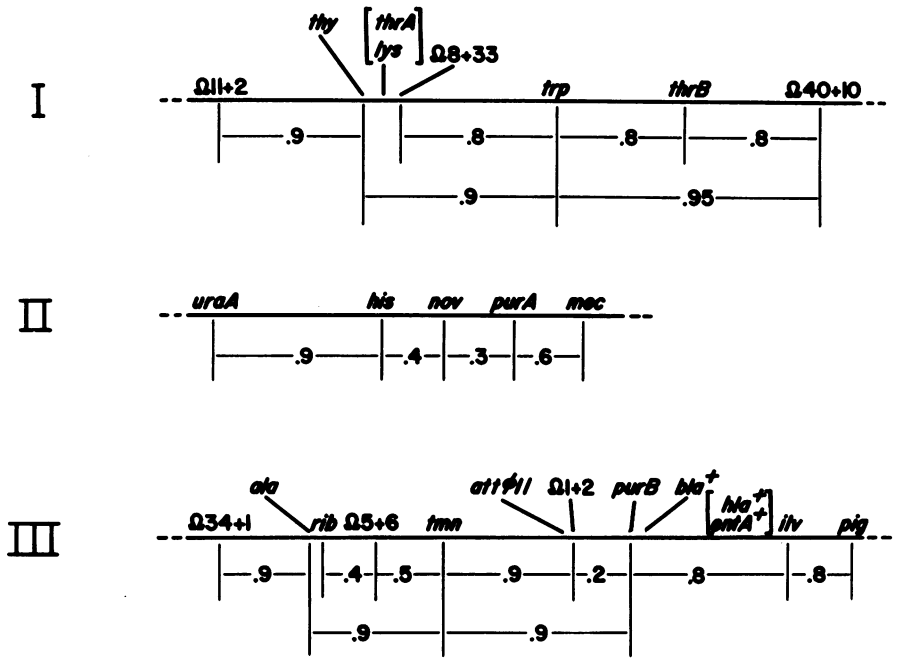


FIG. 1. *The three known linkage groups of the S. aureus chromosome. Distances between markers reflect averages of several crosses, and are expressed as 1 − estimated cotransfer frequencies. Some intermarker map distances have been omitted for clarity. The markers shown and the phenotypes which they confer in instances where the nomenclature is unique to S. aureus are: Ω1, Ω5, Ω8, Ω11, Ω34, and Ω40, separate insertion sites of Tn551 (the notation Ω11 + 2, for example, defines the site of Ω11[Chr::Tn551] and two other insertions of Tn551 at the same site); attφ11, attachment site of prophage φ11; bla+, β-lactamase-mediated penicillin resistance; entA+, enterotoxin A; hla+, α-toxin; mec, methicillin resistance; nov, novobiocin resistance; pig, pigmentation; tmn, resistance to tetracycline and minocycline; uraA141 formerly was pyr-141. (modified from references 13, 15, 16, and 18).*

missive temperature, virtually all of the isolates recovered possess Tn*551* inserted into the only available thermoresistant replicon—the host chromosome. This means of efficiently isolating Tn*551* chromosomal insertions provided not only the means of searching for unique insertion sites of Tn*551*, but also the means of easily identifying insertions that result in auxotrophy as well as Em$^r$. This report presents the results of studies to define the auxotrophic mutations resulting from Tn*551* insertion and the relationship of the majority of these sites to the previously known linkage groups.

## MATERIALS AND METHODS

**Bacteria.** The strains of *S. aureus* used in this study, with their origins and genotypes, are given in Table 1. Strain ISP479, derived as a spontaneous cadmium-resistant (Cd$^r$) revertant of RN-1478(pRN3032), carries *repA36* (responsible for thermosensitivity); although carrying the *bla-401* mutation, the plasmid still encodes for sufficient β-lactamase to readily appear Pc$^r$ on starch agar containing inducer. In addition, the plasmid encodes for resistance to several heavy metal ions. When grown on complete defined synthetic agar (17) lacking individual components, strain ISP479 requires niacin, thiamin, phenylalanine, glycine, arginine, cystine, proline, and glutamic acid; omission of aspartic acid results in suboptimal growth. These growth requirements are representative of the wild-type strain 8325.

**Culture media and reagents.** The composition of complete defined synthetic agar was modified by omitting individual amino acids, purines, and pyrimidines and adding antibiotics as needed (1, 16) to prepare media for the selection and scoring of mutants and recombinants. Antibiotic-resistant phenotypes were scored and selected in some experiments by using brain heart infusion agar containing the appropriate concentration of an antibiotic. Bacto-agar (Difco), rather than Noble agar (17), was used in all media. When needed for the growth of certain isolates, riboflavin was added to complete defined synthetic agar at 1 mg/liter. The Pc$^r$ and Cd$^r$ phenotypes were scored as described previously (18). All media were routinely supplemented with thymine, adenine, guanine, cytosine, and uracil (17), unless a specific omission was required for scoring or selective purposes.

**Transformations and transductions.** The preparation of DNA for transformation and the transformations were performed exactly as described previously (16). Transduction was performed by the method of Proctor and Kloos (19), but with sodium citrate added to the transduction suspension and the selective

TABLE 1. *Designation, genotype, and origins of derivatives of S. aureus 8325*

| Stock no. | Genotype | Origin and references |
|---|---|---|
| ISP5 | 8325 *thy-101 thrB106 ilv-129 pig-131* | 17 |
| ISP7 | 8325 *thy-101 his-116 trp-103 pig-131* | 17 |
| ISP8 | 8325-4 *pig-131* | 20 |
| ISP86 | 8325 *uraA141 hisG15 nov-142 purA102 pig-131* | 10 |
| ISP374 | 8325 Ω40[Chr::Tn*551*] *pig-131 tmn-3106* | 16 |
| ISP479 | 8325-4(pI258 *bla-401 mer-14 repA36*) *pig-131* | Cadmium-resistant revertant of RN1478(pRN3032) |
| ISP514 | 8325-4 *pig-131 trp-159*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP515 | 8325-4 *pig-131 trp-162*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP516 | 8325-4 *pig-131 trp-163*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP517 | 8325-4 *pig-131 trp-164*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP518 | 8325-4 *pig-131 trp-165*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP519 | 8325-4 *pig-131 trp-166*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP520 | 8325-4 *pig-131 trp-167*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP521 | 8325-4 *pig-131 trp-168*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP522 | 8325-4 *pig-131 trp-169*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP523 | 8325-4 *pig-131 trp-170*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP538 | 8325-4 *pig-131 aux-188*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP539 | 8325-4 *pig-131 pdx-189*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP546 | 8325-4 *pig-131* Ω1004[Chr-::Tn*551*] | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP565 | 8325-4 *pig-131 rib-206*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP580 | 8325-4 *pig-131 ilv-230*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP584 | 8325-4 *pig-131 pur-231* Ω1023[Chr::Tn*551*] | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP585 | 8325-4 *pig-131 uraB232*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP586 | 8325-4 *pig-131 uraB233*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP598 | 8325-4 *pig-131 rib-244*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP603 | 8325-4 *pig-131 ala-254*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP623 | 8325-4 *pig-131 his-257*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP637 | 8325 *thy-101 thrB106 ilv-129 pig-131 uraB232*::Tn*551* | ISP5 × ISP585[a] |
| ISP641 | 8325-4 *pig-131 pur-262* Ω1022[Chr::Tn*551*] | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP642 | 8325-4 *pig-131 trp-263*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP672 | 8325-4 *pig-131 tyrA281*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP673 | 8325-4 *pig-131 tyrB282*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP681 | 8325 *thy-101 thrB106 ilv-129 pig-131 tyrB282*::Tn*551* | ISP5 × ISP673[b] |
| ISP686 | 8325-4 *pig-131 metC287*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| ISP691 | 8325-4 *pig-131 metB292*::Tn*551* | This study; Em$^r$ isolate of ISP479 at 43°C |
| RN32 | 8325-3 Ω11[Chr::Tn*551*] *pig-131* | 11 |
| RN1478(pRN3032)[b] | 8325-4(pI258 *bla-401 cad-52 mer-14 repA36*) *pig-131* | 12 |

[a] ISP5 transformed with DNA from ISP585 or ISP673.
[b] pRN3032 was incorrectly identified as pRN3037 in reference 13.

medium (6). The cotransfer frequencies by transformation of two markers, A and B, have been expressed as the estimated cotransfer frequencies (17).

**Isolation of chromosomal insertions of Tn*551*.** After overnight growth at 30°C on brain heart infusion agar, about $5 \times 10^5$ colony-forming units of ISP479 were spread onto each of several plates of brain heart infusion agar containing 20 μg of erythromycin per ml. After incubation at 43°C for 18 to 24 h, each plate contained between 100 and 200 colonies; only 1 or 2% of these colonies were Pc$^r$ and Cd$^r$ at 43°C. By picking these colonies, streaking them for isolation on brain heart infusion agar containing erythromycin (20 μg/ml), and incubating these plates at 43°C, isolates were recovered that were Em$^r$ at 43°C. These isolates were invariably devoid of any additional phenotypic change and are referred to hereafter as silent insertions of Tn*551*. In the majority of experiments, the populations of colonies recovered from ISP479 at 43°C on brain heart infusion agar containing erythromycin were replicated directly onto complete defined synthetic agar containing 20 μg of erythromycin per ml and lacking one or more nutrients that were nonessential for the growth of ISP479. The master and replica plates were incubated at 43°C for 18 to 24 h, after which presumed auxotrophic mutants were scored (by their failure to grow well on the complete defined synthetic agar), picked from the master plate, and struck for isolation on brain heart infusion agar containing erythromycin (20 μg/ml); the streak plates were incubated at 43°C for 24 h. A single colony from each streak plate was then used to prepare a stock culture. Each culture was subsequently characterized for Em$^r$ at 43°C, for auxotrophy on the appropriate complete defined synthetic agar, and for Pc$^s$ and Cd$^s$.

Silent chromosomal insertions of Tn*551* are identified by the symbol Ω followed by an isolation number and the notation [Chr::Tn*551*], and auxotrophic mutations induced by Tn*551* are identified by gene symbol and allele number, followed by the notation ::Tn*551*. This nomenclature is in accordance with the recommendations of Campbell et al. (2).

## RESULTS

**Attempts to isolate additional silent Tn*551* insertions.** The objective in undertaking this study was to define additional regions of the chromosome occupied by Tn*551* without regard for the phenotype of strains carrying these insertions and to use these additions to

extend the chromosome map of *S. aureus* 8325. Accordingly, mutants were isolated from ISP479 that were Em$^r$ at 43°C; their Pc$^s$ and Cd$^s$ phenotypes were confirmed, and then the chromosomal region occupied by Tn*551* was defined in each instance. In repeated experiments, the selection of Em$^r$ mutants of ISP479 at 43°C without further consideration of their auxotrophy resulted only in the recovery of mutants in which Tn*551* was inserted at sites previously so defined (Fig. 1). The only exception was the isolation of ISP546, in which Tn*551* was inserted about midway between *purB110* and *ilv-129* (data not shown). In view of these results, all subsequent experiments were directed toward the isolation of mutants in which Tn*551* insertions resulted in auxotrophy.

**Isolation of auxotrophs resulting from Tn*551* transposition.** A series of experiments was performed to isolate mutants of ISP479 that were Em$^r$ at 43°C and also auxotrophic as a consequence of Tn*551* transposition. The vast majority of these Em$^r$ isolates were Pc$^s$ and Cd$^s$ and exhibited clear-cut auxotrophic characteristics on complete defined synthetic agar. An occasional isolate, although auxotrophic, remained Cd$^r$ and Pc$^r$; when the Em$^r$ of these strains was transformed to an appropriate recipient, the Em$^r$ and auxotrophic phenotypes (but not Cd$^r$ or Pc$^r$) were cotransferred at unity. Cleared lysates of Em$^r$ auxotrophs of ISP479 that retained Cd$^r$ and Pc$^r$ revealed a species of plasmid DNA that comigrated with the pI258 plasmid of ISP479 during agarose gel electrophoresis. This aspect of Tn*551* transposition from the pI258 plasmid has not been studied further, but the limited evidence indicates that the Cd$^r$ and Pc$^r$ of these isolates are due to plasmid-borne markers that remained autonomous after Tn*551* transposition.

Table 2 summarizes the Tn*551*-induced auxotrophic mutants that were retained for further study. This distribution of phenotypes only indirectly reflects the frequency distribution of Tn*551*-induced auxotrophs that occur in strain ISP479 on erythromycin at 43°C. The first experiment was performed by replicating isolates of ISP479 that were Em$^r$ at 43°C onto complete defined synthetic agar lacking tryptophan, threonine, purines, pyrimidines, pantothenic acid, and biotin. Among 131 presumed auxotrophic Em$^r$ isolates that were examined, 105 (80%) were Trp$^-$, 14 were Thr$^-$, 3 were Pur$^-$, 1 exhibited a weak pyridoxal response, 1 was an unidentified auxotroph, and 7 were prototrophic. Subsequent experiments were performed with tryptophan added to the synthetic agar to avoid the repeated isolation of Trp$^-$ mutants. Among the other phenotypes shown in Table 2, the Lys$^-$, Met$^-$, and Tyr$^-$ mutants proved to be readily isolated. In contrast, repeated attempts to isolate Ser$^-$, Thy$^-$, or Pan$^-$ mutants have been unsuccessful. The Ala$^-$, Aux$^-$, His$^-$, and Pdx$^-$ mutants were also rare, with only one example of each having been recovered; however, stringent attempts to isolate Ala$^-$ and His$^-$ mutants were not made.

TABLE 2. *Summary of phenotypes of Tn551-induced auxotrophic mutants of S. aureus 8325-4*

| Phenotype[a] | No. retained for study |
|---|---|
| Ala$^-$ | 1 |
| Aux$^-$ | 1 |
| His$^-$ | 1 |
| Ilv$^-$ | 4 |
| Lys$^-$ | 8[b] |
| Met$^-$ | 8[b] |
| Pdx$^-$ | 1 |
| Pur$^-$ | 11[b] |
| Rib$^-$ | 2 |
| ThrA$^-$ ThrB$^-$ | 23[b] |
| ThrC$^-$ | 3 |
| Trp$^-$ | 11[b] |
| Tyr$^-$ | 8[b] |
| Ura$^-$ | 8 |

[a] Aux$^-$, Unknown auxotrophic requirement for growth on complete defined synthetic agar; ThrA$^-$, requirement for either homoserine or threonine; ThrB$^-$, requirement for threonine; ThrC$^-$, requirement for either homoserine plus lysine or threonine plus lysine.

[b] See text; these are commonly observed classes of Tn*551*-induced auxotrophs whose incidence is higher than that reflected by these numbers.

Several experiments were performed in attempts to change the distribution of Tn*551*-induced auxotrophs isolated or to isolate additional classes of auxotrophs. Neither the use of logarithmically growing cells nor the use of UV-irradiated cells of ISP479 as inocula for brain heart infusion agar containing erythromycin (20 μg/ml) at 43°C had a detectable effect on the pattern of auxotrophs obtained. Because it was thought that the very high proportion of Trp$^-$ mutants might reflect an inadvertant accumulation of *trp*::Tn*551* mutations in the ISP479 stock before selection on erythromycin at 43°C, three clones of ISP479 (isolated at 30°C and shown to possess the autonomous plasmid) were prepared. However, all three clones yielded a very high proportion of Trp$^-$ mutants at 43°C on erythromycin.

**Trp$^-$, Ala$^-$, Rib$^-$, Ilv$^-$, and His$^-$ mutants.** DNAs were extracted from ISP514 and ISP515 (both Trp$^-$), ISP565 and ISP598 (both Rib$^-$), ISP603 (Ala$^-$), ISP623 (His$^-$), and ISP580 (Ilv$^-$) and used to transform appropriate multiply marked derivatives of strain 8325 to identify the sites occupied by Tn*551* in these mutants. In every case, these Tn*551*-induced auxotrophic

mutations occurred in the same regions of the chromosome previously identified by nitrosoguanidine-induced auxotrophs of the same phenotypes (Fig. 1). The Em$^r$ transformants exhibited the same auxotrophic requirements as the donor strains, confirming the identity of the Tn*551* insertion sites and the sites responsible for auxotrophy in these mutants.

The availability of several *trp*::Tn*551* mutations provided the opportunity to determine whether there was any diversity among them; such diversity would be indicative of several different Tn*551* insertion sites affecting the *trp* operon, as opposed to repeated isolations of Trp$^-$ mutants in which Tn*551* was inserted into an identical chromosome site. Accordingly, the growth responses and reversion frequencies of 11 Tn*551*-induced Trp$^-$ mutants were determined. The results (Table 3) show that the site occupied by Tn*551* was not always identical among the Trp$^-$ mutants, and that the frequency of precise excision (8) of Tn*551* varied from one site to another.

**Lys$^-$ and Thr$^-$ mutants.** In several experiments, selection was made for Em$^r$ isolates of strain ISP479 at 43°C that failed to grow on complete defined synthetic agar devoid of threonine and lysine. Mutants exhibiting four readily identifiable phenotypes were recovered from these experiments: Lys$^-$ mutants, ThrA$^-$ mutants, ThrB$^-$ mutants, and a new class (designated ThrC$^-$) that specifically required either threonine plus lysine or homoserine plus lysine for growth. When representative Lys$^-$ (three examined), ThrA$^-$ (one examined), and ThrB$^-$ (three examined) mutants were analyzed by transformation, the Tn*551* insertion sites were shown to map in regions of the chromosome already known to be occupied by *lys*, *thrA*, and *thrB* mutations (Fig. 1). The ThrC$^-$ mutants possessed Tn*551* insertions located between *thy-101* and *trp-103*, with cotransformation frequencies of *thy-101* and *thrC*::Tn*551* ranging from 0.85 to 0.77. In all instances examined, the phenotypes of these Lys$^-$, ThrA$^-$, ThrB$^-$, and ThrC$^-$ mutants were transformed at unity with Em$^r$. The detailed linkage relationship of the *thrC* sites to *lys* and *thrA* has not been analyzed, and the enzymatic basis of the *thrC* mutation has not been investigated. There was no evidence that *thrC*::Tn*551* mutations segregated recombinants that were ThrA$^-$, Lys$^-$, or Em$^r$ when they were used as either donors or recipients by transformation. The ThrC$^-$ mutants appear to be the result, therefore, of a specific Tn*551* insertion into the chromosome.

**Tyr$^-$ mutants.** A single isolation experiment yielded seven Tn*551*-induced Tyr$^-$ mutants. Preliminary transformation experiments demonstrated that all of them carried Tn*551* linked to *thy-101* in linkage group I, and that all but one of these was linked to *thrB106*. The insertion sites for the majority of these *tyr*::Tn*551* mutations are represented by *tyrA281*::Tn*551*. An analysis of this locus (Table 4) by transformation placed *tyrA281*::Tn*551* between *thy-101* and *trp-103*. The relationship of the *tyrA* mutational sites to other genetic elements known to lie between *thy-101* and *trp-103* has not been investigated.

TABLE 3. *Growth responses and reversion frequencies of trp::Tn551 mutants of strain 8325-4*

| Stock no. | Growth on: L-Tryptophan | Growth on: Anthranilic acid | Growth on: Indole | Frequency of reversion[a] |
|---|---|---|---|---|
| ISP514 | + | − | − | $2.5 \times 10^{-7}$ |
| ISP515 | + | − | − | $4.2 \times 10^{-7}$ |
| ISP516 | + | − | − | $6.4 \times 10^{-9}$ |
| ISP517 | + | − | − | $<5.2 \times 10^{-9}$ |
| ISP518 | + | − | − | $8.2 \times 10^{-9}$ |
| ISP519 | + | − | − | $<2.7 \times 10^{-9}$ |
| ISP520 | + | + | + | $<1.4 \times 10^{-9}$ |
| ISP521 | + | − | + | $1.9 \times 10^{-9}$ |
| ISP522 | + | − | − | $<2.1 \times 10^{-9}$ |
| ISP523 | + | − | − | $4.1 \times 10^{-9}$ |
| ISP642 | + | − | − | $3.4 \times 10^{-9}$ |

[a] Frequency of Trp$^+$ revertants per colony-forming unit determined on complete defined synthetic agar devoid of L-tryptophan. All revertants were Em$^s$.

Unlike the *tyrA* mutations, *tyrB282*::Tn*551* showed no evidence of genetic linkage to *thrB106*. Preliminary transformations placed this *tyr* mutation to the left of *thy-101* in a region also occupied by Ω11[Chr::Tn*551*] (Fig. 1). The relationship of *tyrB282*::Tn*551*, Ω11[Chr::Tn*551*] and *thy-101* was analyzed by transforming ISP681 with DNA from RN32; selection was for Thy$^+$, Tyr$^+$, and Thy$^+$ Tyr$^+$, and the resulting transformants were scored for the unselected markers. Em$^r$ among these recombinants could be attributed to the presence of either *tyrB282*::Tn*551* or Ω11[Chr::Tn*551*]; consequently, it was not possible to analyze the incidence of Thy$^+$ transformants that had acquired or lost Ω11[Chr::Tn*551*] but had retained the recipient *tyrB282*::Tn*551* marker. The results of this experiment (Table 5) confirm that *tyrB282*::Tn*551* is on the extreme left of linkage group I. Figure 2 summarizes the linkage relationships of the *tyrA281*::Tn*551*, *tyrB282*::Tn*551*, Ω11[Chr::Tn*551*], *thy-101*, and *trp-103* markers based on the data presented in Tables 4 and 5.

**Met$^-$ mutants.** Eight Tn*551*-induced Met$^-$ mutants recovered from ISP479 were analyzed further. Growth responses placed all mutants into two groups identified as MetB$^-$ and MetC$^-$

by Harmon et al. (in reference 5), as follows: the mutant carrying *metB292*::Tn*551* grew well when supplied with exogenous methionine, homocysteine, or homoserine, but did not respond to cystathionine; the remaining seven mutants, carrying *metC*::Tn*551* mutations, responded well to all of the above compounds. DNAs from these eight Met$^-$ mutants were used to transform ISP5; selection was for Em$^r$ only, and these transformants were scored for Thy, Thr, and Ilv. All of these Met$^-$ mutants were thus shown to have Tn*551* inserted between *thy-101* and *thrB106*, and all exhibited between 31 and 41% cotransformation of Em$^r$ and Thy$^+$ (data not shown). To obtain more detailed data on the location of these sites, DNA from ISP686 (MetC$^-$) and ISP691 (MetB$^-$) was used to transform ISP7, with selection for Thy$^+$, Trp$^+$, and Thy$^+$ Trp$^+$. The results obtained with ISP691 (Table 6 and Fig. 3) place *metB292*::Tn*551* between *thy-101* and *trp-103*. Virtually identical results were obtained with ISP686 DNA (data not shown).

**Ura$^-$ mutants.** Whether induced by nitrosoguanidine (17) or by Tn*551* transposition, all known pyrimidine-requiring mutants of strain 8325 respond to exogenous uracil but not to cytosine. The nitrosoguanidine-induced *uraA141* (formerly designated *pyr-141*; 17) occupies the left extremity of linkage group II (Fig. 1). In contrast, three Tn*551*-induced Ura$^-$ mutants that were mapped in this study have Tn*551* inserted on the right extremity of linkage group I. The latter have been designated *uraB* to distinguish them from *uraA141*. Table 7 shows the results of a transformation between DNA from ISP374 and strain ISP637 that establishes the relative order of the *thrB106*, Ω40[Chr::Tn*551*], and *uraB232*::Tn*551* loci. The *uraB220*::Tn*551*

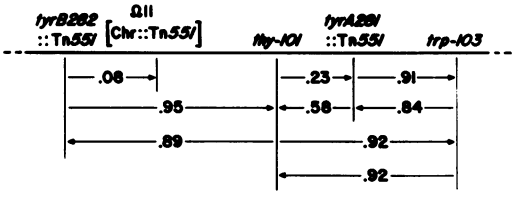


FIG. 2. *Linkage relationships among the tyrB282::Tn551, Ω11[Chr::Tn551], thy-101, tyrA281::Tn551, and trp-103 markers. Numerical values are 1 − estimated cotransfer frequencies calculated from the analyses of unselected markers shown in Tables 4 and 5. Arrows point from the selected to the unselected markers.*

TABLE 4. *Analysis of the thy-101, tyrA281::Tn551, and trp-103 determinants by transforming strain ISP7 (Thy$^-$ Em$^s$ Tyr$^+$ Trp$^-$) with DNA from strain ISP672 (Thy$^+$ Em$^r$ Tyr$^-$ Trp$^+$)*[a]

| Phenotype (no. scored) | % Recombinants detected in class[b]: | | | | | | | Frequency[c] of: | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | Reversion | Transformation |
| Thy$^+$ (772) | 23.1 | 69.3 | 7.3 | | | | 0.4 | 1.6 | 311 |
| Em$^r$ (918) | | 36.7 | 5.5 | 53.8 | | 4.0 | | 4.8 | 426 |
| Trp$^+$ (781) | | | 8.2 | | 83.7 | 7.8 | 0.3 | 38.7 | 359 |
| Thy$^+$ Trp$^+$ (58) | | | 96.6 | | | | 3.4 | <1.6 | 31 |

[a] See Table 1 for genotypes.

[b] Classes: A, Thy$^+$ Em$^s$ Trp$^-$; B, Thy$^+$ Em$^r$ Trp$^-$; C, Thy$^+$ Em$^r$ Trp$^+$; D, Thy$^-$ Em$^r$ Trp$^-$; E, Thy$^-$ Em$^s$ Trp$^+$; F, Thy$^-$ Em$^r$ Trp$^+$; G, Thy$^+$ Em$^s$ Trp$^+$.

[c] Expressed as transformants recovered per $10^9$ colony-forming units; $3.1 \times 10^9$ colony-forming units per ml of the transformation suspension and excess DNA were used.

TABLE 5. *Analysis of the tyrB282::Tn551, Ω11[Chr::Tn551], and thy-101 determinants by transforming strain ISP681 (Em$^r$ Tyr$^-$ Thy$^-$) with DNA from strain RN32 (Tyr$^+$ Em$^r$ Thy$^+$)*[a]

| Phenotype (no. scored) | % Recombinants detected in class[b]: | | | | | Frequency[c] of: | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | Reversion | Transformation |
| Thy$^+$ (648) | 88.6 | 11.4 | | | 0 | <2.4 | 4,408 |
| Tyr$^+$ (1,321) | | 4.6 | 7.9 | 87.5 | 0 | 2.4 | 8,986 |
| Thy$^+$ Tyr$^+$ (847) | | 99.7 | | | 0.3 | <2.4 | 403 |

[a] See Table 1 for genotypes.

[b] Classes: A, Thy$^+$ Em$^r$ Tyr$^-$, was scored only on the basis of Tyr$^-$, and includes transformants possessing and lacking Ω11[Chr::Tn*551*]; B, Thy$^+$ Em$^r$ Tyr$^+$; C, Thy$^-$ Em$^s$ Tyr$^+$; D, Thy$^-$ Em$^r$ Tyr$^+$; E, Thy$^+$ Em$^s$ Tyr$^+$.

[c] Expressed as transformants recovered per $10^9$ colony-forming units; $2.1 \times 10^9$ colony-forming units per ml of the transformation suspension and excess DNA were used.

and *uraB233*::Tn*551* loci were indistinguishable from *uraB232*::Tn*551* by transformation. The results of Table 7 are summarized in Fig. 4. To exclude the possibility that the *uraA141* and *uraB*::Tn*551* loci were allelic, ISP86 was transformed with DNA from ISP586; $His^+$ and $Em^r$ transformants were selected for separately. When more than 5,000 $His^+$ transformants were tested for the cotransformation of $Em^r$, none was detected; likewise, 253 $Em^r$ transformants remained $His^-$. Because the *uraA141* and *hisG15* markers cotransform about 10% of the time (Fig. 1), it is clear that *uraA* and *uraB* are distinct loci on the *S. aureus* chromosome and are not amenable to cotransformation.

**$Pur^-$ mutants** Eleven Tn*551*-induced $Pur^-$ mutants were recovered during the course of several experiments. These mutants exhibited a diversity of responses to adenine, guanine, and purine precursors. With two exceptions, it was not possible to identify the sites of Tn*551* insertion in these $Pur^-$ mutants. Moreover, with these same two exceptions, the $Em^r$ and $Pur^-$ phenotypes were cotransformed at unity to recipient strains. The two exceptions to the foregoing were ISP584, which carried the *pur-231* mutation, and ISP641, which carried the *pur-262* mutation. Both strains were $Em^r$ and $Pur^-$; DNAs from both strains gave rise to $Em^r$ transformants, but these transformants were $Pur^+$. Consequently, the site of Tn*551* insertion and *pur* do not coincide in these strains. This conclusion was reinforced by transducing strains ISP584 and ISP641 with phage $\phi$11/ISP8 with selection for $Pur^+$. ISP641 gave rise to high and equivalent numbers of $Pur^+$ colonies from both the control and transduction suspensions, and these were not characterized further because of this high reversion frequency. From ISP584, only three revertants on two control plates and 85 $Pur^+$ transductants on four experimental plates were recovered. All of the transductants proved to be $Pur^+$ and $Em^r$, confirming that the Tn*551* insertion site was distinct from the *pur* site in this strain. With these observations in mind, strain ISP584 was defined as 8325-4 *pig-131 pur-231* Ω*1023*[Chr::Tn*551*], and strain ISP641 was defined as 8325-4 *pig-131 pur-262* Ω*1022*[Chr::Tn*551*]; the Ω elements define the silent insertions of Tn*551*. Both Tn*551* insertion sites were mapped by three-factor transformations by using DNA from strains ISP584 and ISP641 to transform ISP7. The results (Fig. 5) show that both Tn*551* insertion sites lie between *thy-101* and *trp-103*.

**Miscellaneous mutants.** The two remaining Tn*551*-induced mutants (Table 2) that were examined are ISP538, which carried an unknown auxotrophic requirement (*aux-188*::Tn*551*) that impaired growth on complete defined synthetic agar, and ISP539 (carrying *pdx-189*::Tn*551*), which exhibited a weak growth response to pyridoxal hydrochloride. Both of these mutants grow well on brain heart infusion agar, and their auxotrophic requirements transform at unity with $Em^r$. It was not possible, however, to demonstrate genetic linkage of these Tn*551* insertions and those loci in the known linkage groups by transformation.

## DISCUSSION

The majority of the Tn*551* sites considered in this study have proven to be within the known linkage groups, and this aspect of the results has contributed less to our knowledge of the chro-

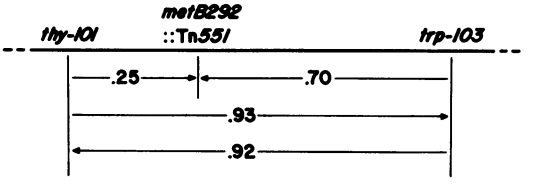


FIG. 3. *Linkage relationships among the thy-101, metB292::Tn551, and trp-103 markers. Numerical values are 1 − estimated cotransfer frequencies calculated from the analyses of unselected markers shown in Table 6. Arrows point from the selected to the unselected markers.*

TABLE 6. *Analysis of the thy-101, metB292::Tn551, and trp-103 determinants by transforming strain ISP7 ($Thy^-$ $Em^s$ $Met^+$ $Trp^-$) with DNA from strain ISP691 ($Thy^+$ $Em^r$ $Met^-$ $Trp^+$)*[a]

| Phenotype (no. scored) | % Recombinants detected in class[b]: | | | | | | Frequency[c] of: | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | Reversion | Transformation |
| $Thy^+$ (431) | 25.3 | 67.8 | 7.0 | | | 0.0 | 3.2 | 278 |
| $Trp^+$ (563) | | | 8.2 | 70.2 | 21.5 | 0.2 | 37.0 | 363 |
| $Thy^+$ $Trp^+$ (67) | | | 100.0 | | | | <1.6 | 27.7 |

[a] See Table 1 for genotypes.

[b] Classes: A, $Thy^+$ $Em^s$ $Trp^-$; B, $Thy^+$ $Em^r$ $Trp^-$; C, $Thy^+$ $Em^r$ $Trp^+$; D, $Thy^-$ $Em^s$ $Trp^+$; E, $Thy^-$ $Em^r$ $Trp^+$; F, $Thy^+$ $Em^s$ $Trp^+$.

[c] Expressed as transformants recovered per $10^9$ colony-forming units; $3.1 \times 10^9$ colony-forming units per ml of the transformation suspension and excess DNA were used.

TABLE 7. *Analysis of the thrB106, Ω40[Chr::Tn551], and uraB232::Tn551 determinants by transforming strain ISP637 (Thr⁻ Ura⁻ Emʳ) with DNA from strain ISP374 (Thr⁺ Emʳ Ura⁺)*[a]

| Phenotype (no. scored) | % Recombinants detected in class[b]: | | | | | Frequency[c] of: | |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | Reversion | Transformation |
| Thr⁺ (233) | 96.1 | 3.9 | | | | 1.9 | 1,666 |
| Ura⁺ (449) | | 2.7 | 52.7 | 44.3 | 0.2 | <1.9 | 1,427 |

[a] See Table 1 for genotypes.

[b] Classes: A, Thr⁺ Emʳ Ura⁻, was scored only on the basis of Ura⁻ and includes transformants possessing and lacking Ω40[Chr::Tn*551*]; B, Thr⁺ Emʳ Ura⁺; C, Thr⁻ Emˢ Ura⁺; D, Thr⁻ Emʳ Ura⁺; E, Thr⁺ Emˢ Ura⁺.

[c] Expressed as transformants recovered per $10^9$ colony-forming units; $2.7 \times 10^9$ colony-forming units and excess DNA were used per ml of the transformation suspension.

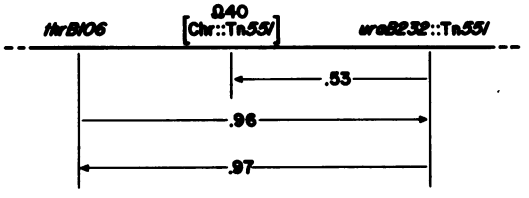


FIG. 4. *Linkage relationships among the thrB106, Ω40[Chr::Tn551], and uraB232::Tn551 markers. Numerical values are 1 − estimated cotransfer frequencies calculated from the analyses of unselected markers shown in Table 7. Arrows point from the selected to the unselected markers.*

mosome of *S. aureus* than had been anticipated. On the other hand, the failure to detect large numbers of new Tn*551* insertion sites that were not linked to the known linkage groups (Fig. 1) adds weight to the view that these linkage groups constitute substantial portions of the entire chromosome. The identification of the *tyrB*::Tn*551* and *uraB*::Tn*551* sites on the left and right extremities of linkage group I (Fig. 1, 2, and 4) is important in two ways. First, this information extends the known limits of linkage group I (although *tyrB*::Tn*551* is only slightly more distal to *thy* than is Ω11[Chr::Tn*551*], and second, both of these markers confer auxotrophic phenotypes whose repair can be selected for in crosses against DNA from silent Tn*551* sites such as Ω34[Chr::Tn*551*]. The latter experiments were not possible by using crosses between silent Tn*551* insertions because of their identical phenotypes.

With the exception of two Pur⁻ mutants induced by Tn*551*, the *pur*::Tn*551*, *aux*::Tn*551*, and *pdx*::Tn*551* sites were not identified relative

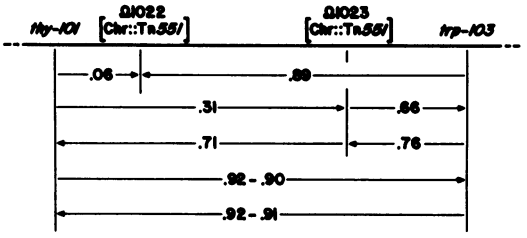


FIG. 5. *Linkage relationships among the thy-101, Ω1022[Chr::Tn551], Ω1023[Chr::Tn551], and trp-103 markers. Numerical values are 1 − estimated cotransfer frequencies calculated from the analyses of unselected markers when ISP7 was transformed with DNAs from ISP584 and ISP641, with selection for Thy⁺, Trp⁺, and Emʳ (the latter with ISP584 only). Upper and lower limits for 1 − estimated cotransfer frequencies for the thy-101 to trp-103 distances are shown. Arrows point from the selected to the unselected markers.*

to the known linkage groups. That the majority of the Tn*551*-induced Pur⁻ mutants are unlinked to the known linkage groups is consistent with the earlier observation that many nitrosoguanidine-induced *pur* mutations are unlinked to these linkage groups (P. A. Pattee, unpublished data). The Pur⁻ isolates ISP584 and ISP641 have Tn*551* sites in linkage group I between *thy* and *trp* (Fig. 5). These two isolates are unique among all other Tn*551*-induced auxotrophs because the Emʳ and auxotrophic phenotypes exhibit no linkage during their transfer by transformation. This absence of linkage was confirmed in ISP584 by showing that Pur⁺ transductants of it remained Emʳ. Although the Emʳ and Pur⁻ characters of these two strains were acquired simultaneously during selection for chromosomal Tn*551* inserts, the Tn*551* and *pur* sites are clearly nonidentical; two possible explanations can be envisaged for this situation: (i) the *pur* mutation occurred spontaneously or in response to heat mutagenesis (3) during selection for Tn*551* transposition, and both events only coincidentally occurred in the same genome; or (ii) the *pur* mutations reflect insertions of Tn*551* that initially resulted in the Pur⁻ phenotype, followed by imprecise excision and transposition of Tn*551* to its present site in each strain, leaving the original site *pur*. Previous studies (13) have shown that Tn*551* excision is usually precise, yet of the two alternatives, ii appears more likely.

The demonstration that the *his, ilv, ala, rib, trp, thrA, thrB,* and *lys* mutations brought about by Tn*551* occupy the same chromosomal regions occupied by nitrosoguanidine-induced mutations of the same phenotypes adds nothing to our understanding of chromosome organization.

However, some additional detail to the known linkage groups has come from the identification of several new Tn*551* insertion sites within the known linkage groups. All known *met*::Tn*551* mutations are clustered between *thy* and *trp*. It seems reasonable to conclude that the Tn*551*-induced *met* mutations represent the *met* gene cluster defined previously (5). In contrast, the Tn*551*-induced *tyr* mutations occupy at least two regions (Fig. 2), one (*tyrB*::Tn*551*) on the left extremity of linkage group I and the other (*tyrA*::Tn*551*) between *thy* and *trp*. The *tyrA* and *tyrB* loci are differentiated only by their positions; they have not been examined for utilization of tyrosine precursors.

In retrospect, it is not surprising that only one undefined auxotroph was isolated during these experiments. The recovery of presumed chromosomal insertions of Tn*551* was accomplished on brain heart infusion agar, an exceedingly rich medium. Replication from brain heart infusion agar onto a defined medium provides little opportunity for the depletion of intracellular levels of nutrients, such as vitamins, whose concentrations required for growth are low. The one Pdx$^-$ mutant isolated exhibited a very poor growth response to pyridoxal hydrochloride, suggesting that this was not the major defect for this Tn*551*-induced auxotroph. In contrast, the Tn*551*-induced Rib$^-$ mutants were readily identified after replication from brain heart infusion agar to complete defined synthetic agar lacking riboflavin. Additional experiments are planned in which the initial recovery of Tn*551* insertions into the chromosome is done on a medium of considerably lower nutritive content.

It is clear from the results of this and previous (13, 18) studies that Tn*551* is capable of occupying a variety of different sites, not only on the chromosome of *S. aureus* but also on other plasmids. In addition to the silent chromosomal insertions (summarized in Fig. 1), Novick et al. (13) have shown that Tn*551* can occupy several different sites on the pI6187 plasmid and cause insertional inactivation of plasmid-borne mercury resistance. Moreover, the results obtained with Tn*551* indicate that insertion into any one site is nonrandom. It also is clear that the insertion of Tn*551* into a given site (as defined by transformation analysis) does not necessarily involve repeated insertion events of an identical nature. Rather, each site consists of a cluster of closely linked insertion sites. The Tn*551*-induced *trp* mutations illustrate the situation well. Among the classes of Tn*551*-induced auxotrophs, the Trp$^-$ mutants are far more numerous than any other class. The *trp* operon of *S. aureus* consists of six adjacent genes (19), at least three of which must be represented by the Tn*551*-induced *trp* mutations of the basis of growth responses (Table 3). Moreover, among those Trp$^-$ mutants responding only to tryptophan (presumably *trpB* mutations; 19), two (ISP514 and ISP515) exhibit reversion frequencies considerably higher than others. Thus, there are at least four different classes of *trp*::Tn*551* mutations. A similar argument can be made with the *met*::Tn*551* mutations, in which two distinguishable phenotypes are apparent from growth responses. These observations raise the possibility that each of the silent insertion sites represented by Ω5, Ω8, and Ω40, where Tn*551* inserts at high frequency, is a cluster of closely linked sites that appear identical by transformation. The nonrandom insertion of Tn*10* into the chromosome of gram-negative bacteria has been well documented, both in terms of the diversity of different regions of the chromosome involved and in terms of specific sites affecting a given operon (4, 7-9). Some other transposition elements also exhibit a nonrandom insertion pattern (summarized in reference 9).

The *thy-trp* region of linkage group I needs to be analyzed in considerably greater detail. This region contains a disproportionately large number of Tn*551* sites, including Ω8[Chr::Tn*551*] (and 33 other identical or similar silent insertions of Tn*551*), together with *thrA, thrC, lys, trp, met, tryA,* Ω1022, and Ω1023. The relative order of these sites (and the genes which they affect) is largely unknown. In particular, the relationship of the *thrA* and *lys* sites to *thrC* may be important. The ThrC$^-$ phenotype (requirement for either threonine or homoserine plus lysine) is unique and difficult to explain by a single enzymatic deficiency. An alternative possibility is that the ThrC$^-$ phenotype is a consequence of Tn*551* insertion in an orientation that has a polar effect on the *thrA*$^+$ and *lys*$^+$ functions. In the opposite orientation, this site could result in either a silent insertion or the *thrA* or *lys* mutation, or the site resulting in *thrC* would be unique and not occupied in the opposite configuration. A more complete knowledge of the relative distances and sequence of the Tn*551* sites involved would greatly aid a more accurate understanding of this situation. Our experience indicates that this analysis would be best performed by generalized transduction, which is better suited to fine-structure genetic analysis than is transformation. The latter has proven to be invaluable for the demonstration of linkage and gene order among elements that are relatively distant from one another, but it lacks the fine resolution necessary to identify gene sequences among closely linked determinants.

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## LITERATURE CITED

1. **Brown, D. R., and P. A. Pattee.** 1980. Identification of a chromosomal determinant of alpha-toxin production in *Staphylococcus aureus*. Infect. Immun. **30:** 36–42.
2. **Campbell, A., P. Starlinger, D. E. Berg, D. Botstein, E. M. Lederberg, R. P. Novick, and W. Szybalski.** 1979. Nomenclature of transposable elements in prokaryotes. Plasmid **2:** 466–473.
3. **Drake, J. W., and R. H. Baltz.** 1976. The biochemistry of mutagenesis. Annu. Rev. Biochem. **45:** 11–37.
4. **Foster, T. J.** 1977. Insertion of the tetracycline resistance translocation unit Tn*10* into the *lac* operon of *Escherichia coli* K12. Mol. Gen. Genet. **154:** 305–309.
5. **Harmon, S. A., J. N. Baldwin, W.-C. Tien, and D. B. Critz.** 1966. Co-transduction of the genetic determinants of synthesis of penicillinase and methionine in *Staphylococcus aureus*. Can. J. Microbiol. **12:** 973–977.
6. **Kasatiya, S. S., and J. N. Baldwin.** 1967. Nature of the determinant of tetracycline resistance in *Staphylococcus aureus*. Can. J. Microbiol. **13:** 1079–1086.
7. **Kleckner, N., R. K. Chan, B.-K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97:** 561–575.
8. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. J. Mol. Biol. **116:** 125–159.
9. **Kleckner, N., D. A. Steele, K. Reichardt, and D. Botstein.** 1979. Specificity of insertion by the translocatable tetracycline-resistance element Tn*10*. Genetics **92:** 1023–1040.
10. **Kuhl, S. A., P. A. Pattee, and J. N. Baldwin.** 1978. Chromosomal map location of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **135:** 460–465.
11. **Novick, R. P.** 1967. Penicillinase plasmids of *Staphylococcus aureus*. Fed. Proc. **26:** 29–38.
12. **Novick, R. P.** 1974. Studies on plasmid replication. III. Isolation and characterization of replication-defective mutants. Mol. Gen. Genet. **135:** 131–147.
13. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P. A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. U.S.A. **76:** 400–404.
14. **Pattee, P. A.** 1976. Genetic linkage of chromosomal tetracycline resistance and pigmentation to a purine auxotrophic marker and the isoleucine-valine-leucine structural genes in *Staphylococcus aureus*. J. Bacteriol. **127:** 1167–1172.
15. **Pattee, P. A.** 1980. Genetic markers of the *Staphylococcus aureus* chromosome, p. 120–123. *In* S. J. O'Brien (ed.), Genetic maps, vol. 1. National Cancer Institute, Bethesda, Md.
16. **Pattee, P. A., and B. A. Glatz.** 1980. Identification of a chromosomal determinant of enterotoxin A production in *Staphylococcus aureus*. Appl. Environ. Microbiol. **39:** 186–193.
17. **Pattee, P. A., and D. S. Neveln.** 1975. Transformation analysis of three linkage groups in *Staphylococcus aureus*. J. Bacteriol. **124:** 201–211.
18. **Pattee, P. A., N. E. Thompson, D. Haubrich, and R. P. Novick.** 1977. Chromosomal map locations of integrated plasmids and related elements in *Staphylococcus aureus*. Plasmid **1:** 38–51.
19. **Proctor, A. R., and W. E. Kloos.** 1970. The tryptophan gene cluster of *Staphylococcus aureus*. J. Gen. Microbiol. **64:** 319–327.
20. **Thompson, N. E., and P. A. Pattee.** 1977. Transformation in *Staphylococcus aureus*: role of bacteriophage and incidence of competence among strains. J. Bacteriol. **129:** 778–788.