Isolation and Characterization of Lambda Transducing Bacteriophages for the su1+ (supD−) Amber Suppressor of Escherichia coli

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Specialized lambda transducing phages for the su1+ (supD−) amber suppressor in Escherichia coli K-12 have been isolated, using a secondary site λl857 lysogen in which we have shown the prophage to be closely linked to su1+. su1+ transducing particles were detected frequently, at 10−4 per plaque-forming unit, in lysates prepared from the secondary-site lysogens. High-frequency transducing lysates were obtained from several independently isolated su1+ transductants and were analyzed by CsCl equilibrium density gradient centrifugation. The transducing phages are defective; marker rescue analysis indicates that the λN gene is not present. In λl857ΔNdSu1+, a bio-type transducing phage, the genes specifying recombination and excision functions have been replaced by bacterial deoxyribonucleic acid.

The su1+ (supD− [43]) nonsense suppressor of Escherichia coli causes translation of the amber nonsense codon UAG as serine (31,41,44). In vitro protein synthesis experiments in several laboratories (4,10,16) have demonstrated that su1+ suppression is mediated by transfer (t) ribonucleic acid (RNA). Specifically, Andoh and Garen (1) and Soll (38) have shown that the relevant suppressor tRNA in this case is a minor species of serine tRNA comprising less than 5% of seryl tRNA.

Direct evidence that su1+ is the structural gene for a serine tRNA with an altered nucleotide sequence which recognizes the UAG codon has not been obtained, primarily because deoxyribonucleic acid (DNA) enriched for the su1+ gene sequence and purified Su1+ tRNA have not been available for biochemical experiments.

We have been interested in analyzing the mechanism of Su1+ suppression. The known nucleotide sequences of E. coli nonsense suppressor tRNAs demonstrate that base changes in more than one position can alter codon recognition to yield a tRNA with nonsense suppressor activity (18,21,29,39,48). Our aim is to discover the pertinent base change in Su1+ tRNA.

We wished to select for a transducing phage which carries the su1+ gene so that, during lytic development of the phage in E. coli cells, transcription from the suppressor gene sequences amplified during phage DNA replication would yield higher levels of Su1+ tRNA. The su1 gene has been mapped near his at 37.5 min on the Taylor and Trotter map of E. coli (14,23), out of range of the regions transducible by λ or φ80. However, several general methods have been developed by which, in principle, almost any E. coli gene can be brought into the range of one of these phages (19,33,36). Shimada et al., (36), using an E. coli mutant deleted for the λ-attachment site, isolated λ lysogens in which the phage had inserted itself at many different secondary chromosomal locations. In one of the lysogens described by these authors, the λ prophage was mapped near his. In this report, we describe the isolation, from this rare lysogen, of specialized λ transducing phages for the su1+ gene.

(This work was part of a dissertation presented by Deborah A. Steege to Yale University, 1974, in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The E. coli K-12 strains used are listed in Table 1. Bacteriophages are listed in Table 2. Figure 1 shows the genetic map positions of relevant markers and Hfr points of origin. In previous publications, the symbol su1 has been used as the name of a gene, i.e., su1+ and su1− have denoted genotypes and Su1+ and Su1− have represented the respective phenotypes (14,22,23). These correspond to the symbols used in the most recent E. coli K-12 map (43) as follows: su1 = supD (the genetic locus which in its active form
mediates UAG suppression); su1+ = supD32 (suppressing form of the su1 gene); su1− = supD+ (non-suppressing form of the su1 gene); Su+ tRNA (suppressor tRNA produced by an Su+ strain; similarly, su2 = supE, su3 = supF, su4 = supC). Su− denotes the suppressor-free phenotype.

DS65 was constructed by crossing KS268 with DS50 (λ). KS268 was grown at 34.5 °C without aeration to a cell density of 2 × 10⁸ per ml, and DS50 (λ) was grown to 4 × 10⁸ per ml at 37 °C with shaking. Hfr and F− cells were mixed in a ratio of 1:10. The mating was allowed to proceed for 2 h at 34.5 °C. At the end of the mating period, the cells were washed with T buffer, serially diluted, and plated on Davis minimal agar plates. His+ (StrF) recombinants were selected at 30 °C.

**Media and growth conditions.** LB broth was as described by Luria and Burrous (28), except that the sodium chloride concentration was reduced to 5 g/liter. Twelve grams of agar (Difco) was added per liter for solid medium. The standard rich medium for preparation of λ phage lysates was λ broth, consisting of 5 g of NaCl, 5 g of tryptone, and 8 g of peptone per liter. The minimal salts medium used for most experiments was TG medium, as described by Garen and Garen (13), except that peptone was omitted. Routinely, a phosphorus concentration of 10 μg/ml was used as KH₂PO₄; carbon sources were added at 0.2%. Solid TG medium included 1.5% Noble special agar (Difco). For liquid cultures, 0.1% Casamino Acids was present, as well as 50 μg of the specific amino acids per ml required by auxotrophic strains. Supplements for plates were as follows: L-amino acids, 10 μg/ml; streptomycin, 100 μg/ml; thiamine hydrochloride, 50 μg/ml; nalidixic acid, 50 μg/ml; and spectinomycin, 67 μg/ml. Kmal medium, derived

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sex</th>
<th>Chromosomal markersa</th>
<th>Source</th>
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<tbody>
<tr>
<td>S26a−</td>
<td>Hfr(Cavalli)</td>
<td>phoA4, rel-1, tonA22, T2a, Su−</td>
<td>A. Garen (15) via E. P. Hoffman</td>
</tr>
<tr>
<td>S26rlε−</td>
<td>Hfr(Cavalli)</td>
<td>phoA4, rel-1, tonA22, T2a, su1+ (supD32)</td>
<td>A. Garen (14) via E. P. Hoffman (23)</td>
</tr>
<tr>
<td>S26rld</td>
<td>Hfr(Cavalli)</td>
<td>phoA4, rel-1, tonA22, T2a, su2+ (supE53) (λ)</td>
<td>A. Garen (14)</td>
</tr>
<tr>
<td>H12R8a</td>
<td>Hfr(Cavalli)</td>
<td>phoA5, rel-1, tonA22, T2a, su3+ (supF34) (λ)</td>
<td>A. Garen (12)</td>
</tr>
<tr>
<td>H12R7a</td>
<td>Hfr(Cavalli)</td>
<td>phoA5, rel-1, tonA22, T2a, su4+ (supC47) (λ)</td>
<td>K. Shimada, strain no. 60 (36). λcI857 genome located near his− derivative of KS268 via nitrosouramidine mutagenesis</td>
</tr>
<tr>
<td>KS268</td>
<td>Hfr(Hayes)</td>
<td>Δatt−,bio, su1− (supD+) (λcI857)</td>
<td>P1u (S26rlε−) × DS60 → His+ Su1+ F+ derivative of KL241</td>
</tr>
<tr>
<td>DS60a</td>
<td>Hfr(Hayes)</td>
<td>Δatt−,bio, his88, su1− (supD+) (λcI857)</td>
<td>Derived from M72 (Brenner) from D. Söll. The trp and lac alleles correspond to trp-Sam and lacY14am, respectively</td>
</tr>
<tr>
<td>DS61a</td>
<td>Hfr(Hayes)</td>
<td>Δatt−,bio, his+, su1+ (supD32), λ8</td>
<td>P2 eductant (26) of KL241, lysogenized with λ</td>
</tr>
<tr>
<td>DS49</td>
<td>F+</td>
<td>arg-47, trp-49, lacZ53, str-150, rel-1, su1− (supD+) (λ)</td>
<td>C. M. Radding</td>
</tr>
<tr>
<td>KL241</td>
<td>F−</td>
<td>arg-47, trp-49, lacZ53, str-150, rel-1, su1− (supD+) (λ)</td>
<td>B. Wilkins</td>
</tr>
<tr>
<td>DS50 (λ)</td>
<td>F−</td>
<td>Δhis, arg-47, trp-49, lacZ53, str-150, rel-1, su1− (supD+) (λ)</td>
<td>L. Soll. Derivative of W3110. The pro, his, and ilv alleles correspond to pro-2, his-29, and ilv-1, respectively. The his and trp alleles are amber mutations (40).</td>
</tr>
<tr>
<td>W3350</td>
<td>F−</td>
<td>gai722, gaiK2, Su−, λ8</td>
<td>KS268 (λcI857) × DS50 (λ) → His+ (StrF) (λcI857)</td>
</tr>
<tr>
<td>C600</td>
<td>F−</td>
<td>thr-1, leu-6, thi-1, lacY1, tonA22, su2+ (supE44), λ8</td>
<td>P1u (S26rlε−) × DS65 → Trp+ Lac+ (Su1+)</td>
</tr>
<tr>
<td>LS289</td>
<td>F−</td>
<td>pro-49, trpS85, trpA9605, his-85, ilv-632, Txs-84</td>
<td>Lysogen carrying su1+ transducing phage obtained by induction of DS68</td>
</tr>
<tr>
<td>DS65</td>
<td>F−</td>
<td>Δatt−, -bio, his+, arg-47, trp-49, lacZ53, str-150, rel-1, su1− (supD+) (λcI857)</td>
<td>Single lysogen carrying an su1+ transducing phage obtained by induction of DS68</td>
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<tr>
<td>DS66a</td>
<td>F−</td>
<td>Δatt−,bio, his+, arg-47, trp-49, lacZ53, str-150, rel-1, su1+ (supD32) (λcI857)</td>
<td>Lysogen carrying su1+ transducing phage obtained by induction of DS68</td>
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<tr>
<td>DS100a</td>
<td>F−</td>
<td>LS289 (λcI857, λdsu1+)</td>
<td>Lysogen carrying su1+ transducing phage obtained by induction of DS68</td>
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<tr>
<td>DS110a</td>
<td>F−</td>
<td>LS289 (λdsu1+)</td>
<td>Lysogen carrying su1+ transducing phage obtained by induction of DS68</td>
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*a Genetic symbols are as given by Taylor and Trotter (43), except for suppressor nomenclature.

**Table 1. Bacterial strains**
TABLE 2. Bacteriophages

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>(\lambda^+)</td>
<td>Ultraviolet induced from S26((\lambda))</td>
<td>Goldberg and Howe (17)</td>
</tr>
<tr>
<td>(\lambda clB57S7)</td>
<td>R. Weisberg</td>
<td>Jacob and Wolman (25)</td>
</tr>
<tr>
<td>(\lambda clir)</td>
<td>R. Devoret via P. Howard-Flanders</td>
<td>Meselson (30)</td>
</tr>
<tr>
<td>(\lambda cl26)</td>
<td>P. Howard-Flanders</td>
<td>Doerfler and Hogness (7)</td>
</tr>
<tr>
<td>(\lambda clB57susN7susN53)</td>
<td>C. M. Radding from E. R. Signer</td>
<td>Radding and Kaiser (34)</td>
</tr>
<tr>
<td>(\lambda csusA32susI27)</td>
<td>C. M. Radding</td>
<td>Kelly and Sunshine (26)</td>
</tr>
<tr>
<td>P1(\nu)</td>
<td>R. C. Wilhelm</td>
<td>Eggen et al. (9)</td>
</tr>
<tr>
<td>P2</td>
<td>R. Calendar</td>
<td>Edgar et al. (8)</td>
</tr>
<tr>
<td>MS2</td>
<td>M. Oeschger</td>
<td></td>
</tr>
<tr>
<td>MS212b</td>
<td>M. Oeschger</td>
<td></td>
</tr>
<tr>
<td>T4N58</td>
<td>R. C. Wilhelm</td>
<td></td>
</tr>
</tbody>
</table>

from Weigle et al. (45), consisted of 10 times diluted M9 salts buffer (5), 1% Casamino Acids, 1% maltose, and 0.01 M MgSO\(_4\). Thiamine hydrochloride and amino acids were added as above for auxotrophic strains. Davis minimal medium (Difco), supplemented with 2 x 10\(^{-4}\) mg of \(\alpha\)-biotin per ml, was used to grow KS268 and its derivatives.

The composition of phage buffer has been given (35). T buffer was prepared as a 10-fold dilution of TG salts buffer.

Cultures were grown with aeration at 37 C in LB or \(\lambda\) broth. \(\lambda clB57\) lysogens were grown at 30 C to prevent induction of the prophage.

**Preparation of lysates.** Stock lysates of \(\lambda cl26\), \(\lambda clir\), P1\(\nu\), T4N58, and the MS2 phase strains were prepared by a plate lysis method (42). \(\lambda\) phage was induced from S26 (\(\lambda\)) with ultraviolet light (500 ergs/mm\(^2\)). Lysates of \(\lambda\) strains which carried the clB57 allele for the temperature-sensitive repressor were prepared by heat induction. The lysogens were grown in \(\lambda\) broth at 30 C to a cell density of 2 to 3 x 10\(^{8}\) per ml, heated at 43 C for 12 min in the presence of 0.01 M MgSO\(_4\), and subsequently shaken at 37 C until lysis was complete, normally 90 min.

**Growth of \(^3\)H-thymine-labeled phage.** \(^3\)H-thymine-labeled phage were prepared as follows: DS100 [LS289 (\(\lambda clB57\), susI\(+\))] strains were grown at 30 C in minimal medium supplemented with isoleucine, valine, and proline. The susI\(+\) prophage was maintained by omitting histidine and tryptophan from the medium, so that suppression of the amber mutations in these operons would be required for growth. At a cell density of 2 x 10\(^8\) per ml, the lysogen was induced as described above. Five microcuries of \(^3\)H-thymine (New England Nuclear) per milliliter was added immediately after induction. To increase the efficiency of isotope incorporation, 250 \(\mu\)g of 2'-deoxyadenosine per ml was added at the same time (2).

**Specialized transduction.** Phage lysates were tested for the presence of susI\(+\) transducing particles by plating with recipient bacterial strains carrying two amber mutations. The recipient strains were LS289, which has amber mutations in the his and trp operons, and KL241, which carries amber mutations in the trp and lac operons. Transductants with wild-type phenotypes for both markers were selected. The SusI\(+\) phenotype of transductants was determined by testing for their ability to support the growth of phage strain T4N58, which carries an amber mutation suppressible by susI\(+\).

Cultures of recipient strains were grown to stationary phase in Kmal medium, supplemented as above. The cells were sedimented by centrifugation, washed, and resuspended in 0.01 M MgSO\(_4\). They were then starved by aeration at 37 C for 45 min. To screen for low frequency transducing (LFT) lysates, the phage particles were first concentrated in 10% polyethylene glycol and 0.5 M NaCl as described Yamamoto et al. (47). They were then preadsorbed at a multiplicity of infection (MOI) of 5 to 10 by mixing with an equal volume of starved recipient bacterial culture and incubating at 32 C for 20 min. This mixture (0.2 ml) was then spread on appropriate selective plates. To titrate the transducing particles in high frequency transducing (HFT) lysates, an MOI of 0.01 was used. After preadsorption, the mixture was serially diluted in T buffer before plating.

**Fig. 1. Genetic map of E. coli K-12 showing relevant markers and the Hfr point of origin for KS268. The outer arc indicates the chromosomal region and point of origin for the F' factor carried by WH1.**
CaCl gradients of HFT phage lysates were assayed directly by preadsorbing 0.1 ml of each fraction (or a 10-fold dilution) to 10⁶ recipient cells and then diluting appropriately for plating.

**Mutant selection.** Strains were mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine, using a procedure similar to that reported by Garen et al. (14), except that log-phase cultures were used. Penicillin selection for histidine auxotrophs was accomplished by following the procedure developed by Curtiss et al. (6), as modified by Hoffman (Ph.D. Thesis, Yale Univ., New Haven, Conn., 1969).

**P1 transduction.** This was carried out with P1Δv as described by Hoffman and Wilhelm (23).

**Test for λ immunity.** λ lysogens were detected by cross-streaking with high-titer lysates of λvir and λc26. Bacterial strains which were lysed by λvir but those that were resistant to λc26 were classified as λ lysogens.

**Rescue of prophage markers by superinfection of single defective lysogens.** Lysogens were grown at 30 C to a density of 2 x 10⁶ to 3 x 10⁶ per ml in λ broth. After concentration by low-speed centrifugation, the cells were resuspended in 0.2 volumes of 0.01 M MgSO₄, and infected with an appropriate mutant phage strain at an MOI of 5. The cells were diluted to their original volume in λ broth containing 0.01 M MgSO₄, after a 20-min preadsorption period. The prophage was heat induced, and the cultures were subsequently shaken at 37 C until lysis was complete.

**Cesium chloride density gradient centrifugation of phage lysates.** Since lambda phage particles band in a CsCl equilibrium density gradient at approximately 1.5 g/cm³, 3-ml gradients of ρ = 1.492 g/cm³ were used to analyze HFT lysates. CsCl 1.95 g was mixed with a solution of 5H-thymine-labeled phage particles and sufficient phage buffer to give 2.52 g of solvent. The samples were centrifuged for 20 h at 42,000 rpm in a type 50 rotor at 4 C. The gradient tubes were punctured, and drops were collected into tubes containing 0.5 ml of phage buffer. Samples of the fractions were assayed for radioactivity, plaque-forming units (PFU), and transducing activity. To each transduction assay 10⁶ PFU of λc857s7 was added to provide any helper functions needed.

**RESULTS**

**Genetic location of the λc857 prophage in strain KS268.** In one of the secondary site lysogens isolated after λc857 infection of an E. coli strain deleted for attl, Shimada et al. (36) located the λ prophage in that region of the chromosome represented between min 38 and 40 on the E. coli genetic map. Since sul maps in the same region, this lysogen (designated no. 60 or KS268) was a potential source of specialized sul⁺ transducing phages. We found KS268 to be Sul⁻, as indicated by its failure to support the growth of T4N58, a phage strain carrying a gene 34 amber mutation which is suppressible by sul⁺.

sul⁺ has been shown to be 6 to 8% co-transducible with his (14). We first attempted to introduce the sul⁺ suppressor gene into KS268 by P1 co-transduction with his. Two independently isolated His⁺ derivatives (DS60 strains) of KS268, which were still lysogenic for λc857, were infected with P1Δv grown on an Sul⁺ donor strain, S26relX-· His⁺ transductants were purified and scored for their Sul phenotype by plating with the amber mutant phage T4N58. Ten of the 83 transductants tested were Sul⁺. All of the Sul⁺ strains (DS61 strains), however, were now sensitive to infection by λc26. Since all 10 of the transductants which had inherited his⁺ and sul⁺ had concomitantly lost the λc857 prophage, this suggested that the prophage in KS268 was linked very closely to, and possibly between, these two genetic markers. Therefore, we reasoned that in strain KS268, the sul gene was possibly within transducing range of λc857. However, it was clear that sul⁺ would have to be introduced using some other method than co-transduction with his.

**Construction of sul⁺ LFT lysogens.** sul⁺ can be used as a direct selective marker in strains carrying nonsense mutations. We achieved this situation by transferring the his⁺-sul⁻ (λc857) region of Hfr KS268 to an appropriate F⁻ strain which had been lysogenized with λ⁺ to prevent zygotic induction. KS268 [Hfr H, sul⁻, ΔattX·bio (λc857)] was mated with DS50(X) [str⁺ Δhis sul⁻ trp⁻ am lac⁻ am (λ⁺)], as described above. His⁺ (Str⁺) recombinants were selected and purified.

When a prophage is induced to enter lytic development, the host cell is killed. The repressor coded for by λ⁺ is temperature resistant and dominant over the temperature-sensitive repressor specified by cI857. Hence, only recombinants carrying the single λc857 genome should be temperature sensitive for growth at 42 C. We therefore scored His⁺ (Str⁺) recombinants for the Lac⁻, Trp⁻, and growth phenotypes. Of 600 recombinants tested, one (DS65) displayed the desired Trp⁻ Lac⁻ temperature-sensitive phenotype.

P1 transduction was used to obtain sul⁺ derivatives of DS65. Trp⁺ transductants were selected on Davis minimal agar and then tested for their Lac phenotype on lactose MacConkey agar. The Trp⁺ Lac⁺ colonies supported the growth of T4N58, which confirmed their Sul⁺ phenotype. Of 150 Lac⁺ Trp⁺ transductants isolated, 32% still carried the λc857 prophage. The group of lysogenic strains (DS68 strains) were used for sul⁺ transducing phage selections. As shown in the following section, some of these proved to yield LFT lysates for sul⁺.

**Isolation of sul⁺ specialized transducing
phages. Lysates prepared from DS68 strains by heat treatment of overnight cultures yielded titers of 10^9 PFU/ml. Several lysates were concentrated and plated on a sterility recipient of 108 lysogens, of a strain carrying the amber mutation in the MS212b. When induced by the defective HFT prophages, the MS212b strain was lysed to yield transducing particles, which were assayed on an RfAm indicator strain. The results indicated that fewer than 1% of the PFU had derived from λsu1+ transducing particles.

The clearest evidence for the defective nature of a transducing phage is the demonstration that a single lysogen is unable to produce viable phage progeny. To do this, His+ Trp+ transductants were purified from a low MOI (0.01) transduction experiment in which LS289 had served as the recipient. Two percent of the transductants carried λ immunity but did not yield phage on a lawn of C600. They were sensitive to λvir, however, which ruled out the possibility that they appeared immune to λC26 superinfection simply because they had become resistant to λ infection.

Identification of the presumptive defective lysogens (DS110 strains) was confirmed by the finding that λsu1+ transducing particles were recoverable after superinfection of the lysogens with λB1857S7. Hence, the λsu1+ transducing phages characterized here are defective phage strains. They carry the temperature-sensitive cB1857 immunity derived from the parental source, λB1857. The genotype is therefore designated λB1857dsu1+.

Deletion mapping by marker rescue. We used marker rescue to determine which genes essential for lytic development had been deleted by the event which produced λdsu1+. When a lambda prophage excises incorrectly from its normal attachment site to generate gal or bio transducing phages, bacterial DNA is generally substituted for phage DNA in the phage genome, as predicted by Campbell (3). In a gal transducing phage, λ DNA to the left of the PP' attachment point is deleted; in a bio phage, λ DNA to the right of the attachment point is deleted. The first gene to the left of PP' essential for lytic development is J, which codes for a protein involved in tail formation. The first essential gene to the right is N. This gene specifies a protein which functions as a positive regulator required for efficient transcription of genes expressed early in lytic development.

In the defective λdsu1+ genome, at least a functional gene J or gene N should be deleted...
(Fig. 2). Conversely, one and only one of the genes should be present and resceivable by superinfecting phage strains carrying amber mutations in these genes. The strains used for marker rescue experiments were λc6susA32susJ27 and λcI857susN7susN53. These carried amber mutations in genes A and J, and two mutations in N, respectively.

Single λdsu1+ lysogens were superinfected with each of the phage mutants. Prophage immunity was lifted by heat induction, and the resulting lysates were plated on Su+ (C600) and Su− (W3350) indicator strains. A+ J+ progeny phage were observed at the level of 2.6%, suggesting that gene J is present in λdsu1+. N+ phage were not detected. In a further experiment (Table 3) in which ΛNN− phage were produced with a burst of greater than 50 from each superinfected lysogen, less than 10¹ N+ progeny were detected per 10⁷ ΛNN− phage particles.

The absence of gene N suggested that λdsu1+ is a bio-type transducing phage (see Fig. 2), which must have derived from a prophage which had been inserted into the bacterial chromosome of KS268 with an orientation of opposite polarity to that which occurs at the normal λ attachment site.

Characterization of HFT lysates. Several lysates were analyzed in greater detail to characterize the heterogeneity among uniquely generated transducing particles. Variation in transducing titers among HFT lysates derived from independently isolated LFT lysogens (see

![Possible origins of defective *SuI* transducing phage lines](image)

**Fig. 2.** The possible origins of defective *suI* transducing particles from low frequency transducing DS68 lysogens. (a) if *λ* has inserted itself such that prophage and flanking bacterial markers have the same relative orientation as occurs at the normal λ attachment locus, then gal-type *suI* transducing phages, in which at least gene J is deleted, result. (b) If the prophage has an opposite orientation, bio-type *suI* transducing particles, which lack at least N, are expected.

<table>
<thead>
<tr>
<th>Table 3. Rescue of λ genes by λdsu1+</th>
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<tr>
<td>Experiments</td>
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<tr>
<td></td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>DS110-1</td>
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<tr>
<td>DS110-2</td>
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<tr>
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<tr>
<td>DS110-1</td>
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* Phage titers in PFU per milliliter.
DISCUSSION

We have undertaken the selection of specialized transducing phages for the sul\(^+\) (supD\(^-\)) gene. In this report we have shown via P1 transduction mapping that the XcI857 prophage (s) in KS268, a rare secondary site lysogen isolated by Shimada et al. (36), is closely linked to sul. This close linkage was further demonstrated by the finding that when suppressor activity was used as the directly selected function to introduce the sul\(^+\) allele into the KS268 derivative, DS65, only 32% of the P1 transductants retained the prophage. These results suggested that sul\(^+\) was within transducing range of XcI857 in this strain.

In lysates prepared by inducing a lysogen in which \(\lambda\) has integrated at its normal attachment site, gal and bio transducing particles are generally detected at \(10^{-4}\) per PFU. In LFT lysates induced from several DS68 isolates, sul\(^+\) transductants appeared at \(10^{-4}\) per PFU. The relatively high ratio of transducing particles to PFU in these experiments may be a consequence of the abnormal location of the prophage. Normal xis-mediated excision from the secondary site might be impaired without affecting the frequency of transducing particle formation via illegitimate recombination events.

The number of sul\(^+\) transducing particles determined for HFT lysates often was equal to the number of PFU. Other investigators have reported transducing titers 10 to 100-fold lower than helper phage titers (19,33). However, when they banded phage lysates in CsCl, the ultraviolet absorbance corresponding to the helper and transducing phage particles often was equal. This suggests that they detected only a fraction of the transducing particles by transduction assays. In contrast, it seems likely that we detect a high percentage of \(\lambda\)dsul\(^+\) particles. In CsCl gradient analysis of HFT lysates, the Xdsul\(^+\) titers obtained from transduction assays correspond to the percentage of the total radioactivity in \[^3\text{H}\]thymine-labeled phage with transducing activity. In early experiments we obtained 10-fold more Sul\(^+\) transductants when we grew the recipient bacterial strain in a maltose minimal medium (Kmal) than when the strain was cultured in glucose-containing LB broth supplemented with 0.2% maltose. Use
of maltose as the sole carbon source would be expected to enhance recovery of transductants. The sensitivity of the cells to infection should be maximized, since a gene in the mal operon codes directly for the λ adsorption site. Moreover, activation of the catabolic pathway should increase intracellular levels of 3′5′-cyclic adenosine monophosphate. This has been suggested to increase the lysogenic response (20,24). Efficient detection of sul\(^+\) transducing particles may also be a consequence of the genetic structure of the λdsu\(^+\) phages obtained thus far. Since a functional N gene is not present, cells singly infected with a transducing phage would not be subject to host killing.

The lack of a functional N gene in λdsu\(^+\) suggests that it is a bio-type transducing phage. If we assume that the excision error which produced the transducing phage occurred as expected from the Campbell model (3), then the transducing phage genome must also lack the phage genes which lie between the P\(^-\) attachment point and N (see Fig. 2). These include the gene which specify the integration and excision functions. λdsu\(^+\) should, therefore, be unable to integrate at the λ attachment locus on the E. coli chromosome. Hence, single lysogens most likely carry the prophage as a plasmid, as described by Signer (37), or as a prophage inserted by homology with bacterial sul locus. Since N function is required for expression of genes involved in the establishment of phage immunity, a plasmid-containing lysogen is not, in fact, immune to superinfection. However, it appears immune when tested by superinfection with a λN\(^+\)cl\(^-\) phage, as we observed when λdsu\(^+\) single lysogens were cross-streaked with λc26. In this case, the N\(^+\) product made by the incoming phage can complement the N defect of the resident phage to provide the gene products needed for establishment of repression.

In principle, all of the methods designed to position genes near the attachment sites for temperate bacteriophages are general and could yield specialized transducing phage strains carrying any E. coli gene. However, all of the procedures rely on genetic selection. In practice therefore, one cannot specify in advance the exact proximity or the relative orientation of a given prophage and the gene of interest. Moreover, the rearrangements involved in the construction of potential low frequency transducing strains are results of illegitimate recombination events (11). These occur rarely and very likely not with equal probability at all sites on participating DNA molecules (27,32). Hence, for any particular gene, not all approaches may yield transducing phages at a detectable frequency.

The approach described in this report, using a secondary site λ lysogen, was only one of several undertaken to attempt the isolation of sul\(^+\) transducing phages. Concomitantly, using the method described by Press et al. (33), fusion products of the two epimorphs F\(^+\)WH1 (his\(^+\) sul\(^+\)) (23) and F\(^-\)trp\(^-\)att\(_{\lambda\text{so}}\) (33) were isolated. Directed transposition strains with F\(^+\)WH1 (his\(^+\) sul\(^+\)) (23) inserted at tonB, near att\(_{\lambda\text{so}}\), were also obtained (19). In addition, we integrated the his transducing phage λc1857st68h80gndhis, described by Wolf and Fraenkel (46), at the his locus in a his\(^-\) sul\(^+\) bacterial strain, as a means of selecting for new transducing phages carrying sul\(^+\). Lysates were prepared from potential LFT strains constructed using each of these approaches and were analyzed for the presence of sul\(^+\) transducing phages. LFT sul\(^+\) transduction was not detected in any of the lysates, whereas the secondary site λ lysogen yielded specialized sul\(^+\) transducing phages with a relatively high frequency, as described in this report.

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