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Isolation of a Specialized Lambda Transducing Bacteriophage Carrying the Beta Subunit Gene for *Escherichia coli* Ribonucleic Acid Polymerase

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A heat-inducible lysis-defective λ prophage has been integrated directly into the *E. coli* chromosome at a site (*bfe*) very closely linked to the ribonucleic acid polymerase mutation *rif*^{d}, a dominant rifampin resistance allele. This unusual lysogen has facilitated the isolation of specialized transducing phages conferring rifampin resistance to sensitive cells, and carrying at least the β subunit gene of RNA polymerase in intact form.

Specialized transducing phages have been isolated for a number of different groups of bacterial genes. Such phage arise when the prophage deoxyribonucleic acid (DNA) excises in a faulty manner from the chromosome and carries with it a portion of the adjacent bacterial DNA (9). These phages have not only provided a source of bacterial genes for the study of gene expression in vitro, but also have greatly facilitated the genetic analysis of specific regions of the chromosome. Although specialized transducing phages can normally be isolated only for those bacterial loci closely linked to the prophage attachment sites, several methods have been developed for the isolation of transducing phages from other regions of the chromosome (4, 21, 29, 30).

The isolation of phages carrying the genes for *Escherichia coli* ribonucleic acid (RNA) polymerase (ribonucleoside triphosphate: RNA nucleotidyl transferase, EC 2.7.7.6) would provide a valuable tool for further genetic and biochemical studies of this enzyme. RNA polymerase, which is responsible for most if not all RNA synthesis from DNA templates, is a complex enzyme having an $\alpha_2\beta\beta'\sigma$ subunit structure (5, 7, 36). Despite more than a decade of intensive study, almost nothing is known about the organization of the genes coding for its several subunits or about the control processes which regulate subunit synthesis in growing cells.

To date, only the position on the bacterial chromosome of the β subunit gene has been firmly established. The antibiotics rifampin and streptolydigin interact specifically with RNA polymerase and inhibit, respectively, transcription initiation (15) and chain elongation (11). Bacterial mutants resistant to these drugs synthesize an RNA polymerase with an altered β subunit (19). These mutations map at 79 min on the standard genetic map (33). Kinetic studies on the synthesis of the β and β′ subunits in vivo have shown that both of these subunits could be synthesized sequentially from the same polycistronic messenger (27). A study on the polarity effects of *rif*^{o} nonsense mutants (2) suggests that the genes for the β and β′ subunits are included in a single operon (R. Hayward and J. Scaife, personal communication). Whether all of the other subunit genes belong to this same operon is not known since mutations in the other subunits have not yet been identified. Assuming that the α, β, β′, and σ subunit genes do constitute a single operon, the amount of DNA needed to code for the complete enzyme is 1.2×10^4 base pairs, or approximately 25% the size of the genome of bacteriophage λ (13). Transducing phages carrying substantially more than this amount of bacterial DNA have been isolated for other regions of the chromosome (25).

Normally, the mutations to rifampin and streptolydigin resistance are recessive to the wild-type alleles (2). Therefore, it is not possible to use these mutations to select for phages carrying the β subunit of RNA polymerase since such phage cannot confer resistance to sensitive

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cells. We have approached this problem in two ways. In the accompanying publication (25) we describe the isolation of ϕ80 transducing phages carrying the *rif*^{r} allele, obtained by initially selecting for phage which transduced the closely linked *supA36* mutation, in the hope that these phage fortuitously carried the unselected *rif*^{r} locus.

Here we report the isolation of defective *rif* transducing phages obtained by a much more direct approach. Direct selection of these phage was made possible by the isolation of a dominant rifampin resistance mutation (*rif*^{d}). These λd*rif*^{d} phage were initially isolated by inducing an unusual λ lysogen in which a heat-inducible lysis-defective prophage has been integrated directly into the chromosome at *bfe*, a site very closely linked to *rif*^{d}.

MATERIALS AND METHODS

Bacterial and phage strains. The source and genotype of each bacterial and bacteriophage strain used are listed in Table 1. All bacterial strains are derivatives of *E. coli* K-12.

Media. Recipes for LB broth, 2xYT broth, R plates, EMBO plates, H-top agar, R-top agar, F-top agar, phage buffer (SM), and M63 minimal plates are described by Miller (28). TYE plate medium contains (per liter) 10 g of tryptone (Difco), 5 g of yeast extract, 8 g of NaCl, 15 g of agar (Difco). T medium contains (per liter) 10 g of tryptone (Difco), and 5 g of NaCl plus 12 g of agar (Difco) for plate medium and 7 g of agar for top agar. Rifampin was obtained from Schwarz/Mann, Orangeburg, N.Y., and was dissolved in 100% methanol prior to addition to the autoclaved medium.

Phage lysates. Stocks of P1*vir*, BF23, and λcI60 were prepared by the plate lysate method (28). R medium was used for P1*vir* and BF23, and T medium for λcI60. The low-frequency transducing lysates were prepared from strain XT113 as follows: 10 independent 1-liter cultures of XT113 were grown at 32 C with vigorous aeration in 2xYT to an optical density at 550 nm (OD_{550}) of 0.5 to 0.7. The cultures were induced by shifting to 42 C for 20 min with vigorous aeration. Incubation was then continued for 3 h at 37 C. Except where noted, all of the subsequent steps were performed at 4 C. The induced cells were pooled and harvested by centrifugation at 15,000 × *g* in a Lourdes continuous flow centrifuge (yield, 25 g of cells wet weight). The frozen cells were added to 25 ml of phage buffer and were lysed by thawing at 37 C. DNase was added to 0.1 μg/ml and the lysate was incubated at 4 C with stirring until there was no further change in viscosity. The lysate was freed of cell debris by centrifugation for 30 min at 15,000 rpm in a Sorvall SS-34 rotor. The phage were further concentrated by centrifugation for 2 h at 35,000 rpm in a Spinco angle 40 rotor. The pellets were suspended in the minimum amount of phage buffer by overnight shaking on a wrist-action shaker. The suspended pellets were pooled to give a phage preparation of approximately 3 ml with a titer of 3×10^8 plaque-forming units (PFU)/ml. The phage were stored over chloroform at 4 C. Stocks of λcI857s7 and high-frequency transducing lysates for the λcI857s7d*rif*^{d} phages were prepared by heat induction of the appropriate lysogens, i.e., H105 (λcI857s7) and H105 (λcI857s7, λcI857s7d-*rif*^{d}). Cultures (5 ml) were grown in LB broth at 32 C to OD_{550} of 0.5 to 0.7 and induced by shifting to 42 C for 20 min with vigorous aeration. Incubation was continued for 3 h at 37 C, at which time the cells were artificially lysed by adding chloroform to saturation and continuing incubation for 10 to 15 min. Cell

TABLE 1. *Bacterial strains*[a]

Designation	Mating type	Genotype	Source
X204	F$^-$	Δ(*gal att*λ *chlA bioA uvrB*) *spcA strA thi bfe argH*	J. Kirschbaum
X205	F$^-$	Δ(*gal att*λ *chlA bioA urvB*) *spcA strA thi argH rif*^{d}	J. Kirschbaum
X217	F$^-$	*gal2 strA*	M. Meselson: strain 28
QD5003	F$^-$	*mel, supF*	J. Greenblatt
E111a	F′	KLF10 *rif*^{d}/*metB ins*394 *trpA*36 *lac*2	J. Kirschbaum
H102	HfrH	*thi*	J. Gross: strain HfrH
H105	HfrKL16	*thi recA1*	B. Low: strain KL16-99
H113	HfrP4X	*thi metB argE* (amber)	D. Ganem: strain E210
H117	HfrKL16	*thi recA1 rif*^{r} (λcI857s7)	J. Kirschbaum
XT113	F	Δ(*gal att*λ *chlA bioA uvrB*) *bfe spcA strA thi argH rif*^{d} (λcI857s7 integrated at *bfe*)	J. Kirschbaum

[a] Phages: λcI857s7 was from J. Greenblatt; λcI60 was from I. Herskowitz; BF23 was from N. Glansdorff. All bacterial strains are derivatives of *E. coli* K-12. Mutant allele names are given according to the convention proposed by Demerec et al. (14), and used by Taylor and Trotter (33). Other abbreviations are *ins*, a mutation which restores normal growth in rich nutrient medium to cells possessing *supA*36 (10), and *rif*^{d}, a dominant rifampin resistance allele (Kirschbaum, Ph.D. thesis).

debris was removed by centrifugation and the lysates were stored over chloroform at 4 C.

Transductions. P1*vir* transductions were performed according to Miller (28). Transductions with λ were done by the method of Signer (31) at a multiplicity of 5 plaque-forming phage per cell. *Rif*^{d} transductants were selected by plating the infected cells in 2.5 ml H-top agar on TYE plates and incubating the plates for 2 h (at 37 C for P1*vir* transductions, and at 32 C for λ transductions). The plates were then overlayed with 2.5 ml of H-top agar containing 1.2 mg of rifampin per ml, to give a final plate concentration of approximately 100 μg/ml. This delayed selection is necessary because the *Rif*^{d} phenotype is fully expressed only after a brief lag period (Kirschbaum, Ph.D. thesis, Harvard University, Cambridge, Mass.).

Prophage curing. Curing of λ lysogens by heat pulse and spontaneous methods, and the measurement of the respective curing rates are described elsewhere (30).

Prophage mapping. Equal volumes of log-phase LB broth cultures of the Hfr donors H105 (λcI857s7, λcI857s7*drif*^{d}) grown at 32 C to about 2×10^8 cells/ml were mixed with an equal volume of a log-phase broth culture of the recipient X217 (λ^+) (2×10^8 cells/ml) and matings were carried out at 32 C for 2 h with gentle aeration. The mating mixtures were diluted 10-fold with 10 mM $MgSO_4$, vortexed, and 0.1-ml samples were spread on galactose minimal medium containing 150 μg of streptomycin per ml. One hundred ten of the Gal$^+$ Str^r recombinants from each cross which appeared at 32 C were gridded into the same selective medium and were scored for *Rif*^{d} at 32 C by replica plating onto TYE plates with and without 100 μg of rifampin per ml. Strain H117 served as the control donor.

RNA polymerase assays. One gram of stationary phase cells was disrupted by sonic treatment, and the RNA polymerase was purified through the initial steps of the Burgess procedure (6), without the addition of DNase. The final ammonium sulfate fraction was assayed for the ability to incorporate [^{3}H] uridine 5'-triphosphate (UTP) into trichloroacetic acid precipitable material. Samples of each extract (20 μliters) were preincubated with rifampin for 20 min at 4 C. Then a complete assay mixture (0.1 ml) containing 150 μg of calf-thymus DNA per ml of labeled UTP (final SA, 1,000 μCi/μm), and 1 mM potassium phosphate to inhibit polynucleotide phosphorylase, was added to the preincubated extracts. Assays were performed for 15 min at 37 C, and the reactions were terminated by the addition of 5% trichloroacetic acid. The precipitates were chilled, collected on Millipore DA filters, washed, dried, and counted in toluene-omnifluor scintillation mix by using a Beckman liquid scintillation counter. The activity of the extracts was dependent on added template.

Insertion of λcI857s7 into bfe. A single colony of strain X205 was grown to saturation in 5 ml of LB broth at 37 C. A 1-ml sample of this culture (4×10^9 cells) was infected at a multiplicity of 10 with λcI857s7 by the method of Shimada, Weisberg, and Gottesman (30). The infected cells were diluted 100-fold into fresh LB broth and were grown to saturation at 32 C to allow complete segregation of the recessive Bfe^r mutants. A 5-ml sample (3×10^{10} cells) was then infected with bacteriophage BF23 and λcI60 multiplicities of 10, and 0.5 ml of these infected cells were spread on each of 10 EMBO plates at 32 C. The survivors were repurified by two cycles of streaking on TYE plates containing 100 μg of rifampin per ml at 32 C. They were subsequently tested for temperature-sensitive growth to eliminate those survivors which were not λ lysogens but merely resistant to λcI60. Fifty of the Bfe^r λ lysogens were heat-pulse cured and the survivors were scored at 37 C for loss of resistance to phage BF23 by spotting approximately 10^7 phage on a cross streak of the cells to be tested on TYE plates.

Purification of λcI857s7drif^{d}18. A 1-liter LB broth culture of H105 (LcI857s7, λcI857s7*drif*^{d}18) was heat induced and cells were harvested 3 h after induction by centrifugation for 15 min at 15,000 × *g* in a Sorvall GSA rotor at 4 C. The cell pellet was suspended in 5 ml of phage buffer and was lysed, treated with DNase, and freed of debris as described above. This crude lysate was then purified by CsCl block-gradient centrifugation (28) followed by centrifugation to equilibrium in a CsCl density gradient (28). The heavier of the two phage bands on the final density gradient contained the λcI857s7*drif*^{d}18 phage, and the DNA was purified from the phage particles by extraction with hot sodium dodecyl sulfate (28).

RESULTS

RNA polymerase rif^d mutation. Mutants of *E. coli* which are resistant to rifampin arise spontaneously at a frequency of approximately 10^{-8} (24). The *rif*^{r} mutations are recessive to the wild-type *rif*^{s} allele since *rif*^{s}/*rif*^{r} partial heterodiploids fail to grow in the presence of high levels of the antibiotic (2). It has been suggested that *rif*^{r} is recessive because in the presence of rifampin, the Rif^s RNA polymerase molecules remain bound to the DNA at the promoter sites and fail to initiate transcription. The Rif^r enzyme molecules are thereby prevented from interacting with the promoter sites and initiating transcription (20).

Mutations to rifampin resistance which are dominant to the *rif*^{s} allele have been isolated independently by Babinet (3) and Kirschbaum (Ph.D. thesis, Harvard University). These *rif*^{d} mutations which occur spontaneously at a frequency of approximately 10^{-9} to 10^{-10} were obtained by selecting for mutants in a population of F'*rif*^{s}/*rif*^{s} Rec$^-$ merodiploids which would grow in the presence of rifampin. A normal recessive *rif*^{r} mutation in one copy of the *rif* locus would not confer resistance due to the presence of the otherwise dominant *rif*^{s} allele. On the other hand, a dominant rifampin-resistance mutation would allow these cells to grow normally in the presence of the drug.

Analysis of the *rif*^{d} mutants obtained in this

fashion has shown that the *rif*^{d} allele is dominant, regardless of whether it is located on the bacterial chromosome or is carried by the F' factor in the partial diploids. The *rif*^{d} mutation results in the synthesis of an RNA polymerase which is completely resistant to rifampin in vitro. In partially purified extracts prepared from stationary phase cultures of an F'*rif*^{d}/*rif*^{s} heterodiploid, the resistant enzyme accounts for at least half of the total activity (Kirschbaum, Ph.D. thesis). Three factor transductional mapping experiments have located the *rif*^{d} allele at 79 min on the *E. coli* chromosome. This marker is 52% cotransducible with *argH*-2 and 40% cotransducible with *purD*, and the relative gene order *arg*H-2: *rif*^{d}: *purD* has been established (Kirschbaum, Ph.D. thesis). Therefore the *rif*^{d} mutation is located in the same region of the chromosome as other mutations which are known to affect RNA polymerase. Studies on the mechanism of dominance of the *rif*^{d} allele are in progress.

Directed insertion of a λ prophage into bfe. Specialized transducing phage can be isolated only for those bacterial markers which are extremely close to the site of an integrated prophage. Therefore, to isolate transducing phage carrying the *rif* region, it is necessary to minimize the distance between the integrated prophage and the *rif* locus. Phage λ normally integrates at a single site on the bacterial chromosome (*att*λ) (33), separated from the *rif* locus by a distance corresponding to one third of the *E. coli* chromosome. However, it has recently been shown that infection of cells which are deleted for *att*λ yields stable lysogens at a reduced frequency, in which the prophage is integrated at a variety of other secondary sites on the *E. coli* chromosome (30). Induction of these lysogens has in many cases resulted in the formation of specialized transducing phage lines which carry those regions of the bacterial chromosome closely linked to the new site of the prophage insertion.

We have used strains carrying a deletion of *att*λ (Δ*att*λ) to direct the insertion of λ into a site on the bacterial chromosome which is not only very closely linked to the RNA polymerase mutation *rif*^{d}, but also allows a convenient selection for the prophage insertion. This locus is *bfe* and is required for the normal synthesis of sites on the cell wall necessary for the adsorption of the E-group colicins and bacteriophage BF23 (8). Mutations in this gene produce a resistant phenotype, and arise spontaneously at a frequency of 10^{-6}. Three factor crosses (Table 2) show that *bfe* and *rif*^{d} are 88% cotransducible, and establish the gene order *argH-bfe-rif*^{d}.

TABLE 2. *Mapping the bfe locus*[a]

Selected	Unselected	No. transductants
Rif^d	Arg$^+$Bfe^r	3
	Arg$^+$Bfe^s	140
	Arg$^-$Bfe^r	39
	Arg$^-$Bfe^s	154

[a] P1*vir* prepared on strain E111a (KLF10 *rif*^{d}/*metB trpA*36 *lac*2 *ins*394) was used to transduce strain X204 (F$^-$ Δ(gal *att*λ *chlA bioA uvrB*) *thi spc strA bef argH*) to *rif*^{d}. The initial *rif*^{d} transductants were gridded into TYE medium containing 100 μg of rifampin per ml, and were scored for the Arg phenotype by replica plating on to glucose-minimal plates containing 1.25 μg of vitamin B_1 and biotin per ml, with and without arginine (30 μg/ml) and for the Bfe phenotype by replica plating on to rich plates seeded with about 10^9 BF23. Incubations were at 37 C.

These results are in good agreement with the data of Jasper et al. (22). A genetic map of the *bfe* region of the chromosome is presented in Fig. 1.

Selecting for the insertion of a λ prophage into the bacterial chromosome at this site is straightforward. The integration of the λ genome into *bfe* represents the insertion of an extensive amount of foreign DNA into the *bfe* site. Synthesis of the normal *bfe* gene product(s) is therefore prevented, and a Bfe^r phenotype results, since these cells are no longer able to adsorb bacteriophage BF23.

Isolation of the directed prophage insertion strain. We chose to construct the transducing phages from λcI857s7, a heat-inducible (32), lysis-defective (16) phage which is particularly useful for the preparation of high-titer phage lysates. This phage was adsorbed to strain X205, which carried Δ*att*λ and *rif*^{d}. After a brief period of cell growth at 32 C to allow complete expression of the recessive Bfe^r phenotype (8), survivors which were Bfe^r λ-lysogens were selected by spreading samples of the infected culture on rich plates seeded with the bacteriophage BF23 and λcI60, a λ clear mutant which kills nonlysogens but fails to grow on cells already lysogenic for phage λ (23). Survivors occurred at a frequency of approximately 10^{-8} and failed to grow at 42 C, as would be expected for cells lysogenic for the heat-inducible λcI857s7 prophage.

The temperature-sensitive, Bfe^r phenotype of these cells could have arisen by either of two events: (i) insertion of the λcI857s7 into the *bfe* locus as desired, or (ii) spontaneous mutation to Bfe^r together with prophage insertion into any other possible secondary attachment site on the bacterial chromosome. To distinguish these two

possibilities, the prophage in these survivors was cured by heat-pulse treatment (34). It is known that heat-pulse curing of the secondary attachment site λcI857 lysogens results in the restoration of the specific gene functions which were inactivated upon integration of the prophage (30). Therefore, in the Bfe^r λ-lysogens in which the λ had become integrated into *bfe*, heat-pulse curing of the prophage would be expected to restore the Bfe^s phenotype. In contrast, in those Bfe^r λ-lysogens which carry a spontaneous mutation to Bfe^r together with the insertion of λ at some unrelated site on the chromosome, heat-pulse curing should yield survivors which are still Bfe^r.

This test showed that approximately 2% of the Bfe^r lysogens yielded Bfe^s survivors after heat-pulse curing. This finding is taken as evidence for the insertion of λcI857s7 into *bfe*, and suggests that the insertion arose at a frequency of approximately one cell in 10^{10} cells originally infected with λ. Experiments on one such unusual lysogen, designated XT113, have shown that the prophage integration into *bfe* produced an extremely stable lysogen. The heat pulse and spontaneous curing frequencies are 3.9×10^{-4} and 7.9×10^{-8}, respectively, and are approximately 10^3-fold lower than the corresponding values for a normal lysogen (30). Furthermore, heat induction of XT113 produced plaque-forming phage at the level of one phage for every 10^4 cells induced. In contrast, induction of normal attachment site λcI857s7 lysogen yields up to 10^3 phage per cell (16).

Isolation of low-frequency transducing lysates. A concentrated phage lysate prepared from XT113 has been analyzed for its ability to transduce bacterial genes located on either side of the site of the prophage insertion (Table 3). The *arg*E marker is transduced at a frequency of 3.6×10^{-4}, whereas the *rif*^{d} locus is transduced at frequencies between 6.4×10^{-5} and 7.5×10^{-5}. Transduction of a *rif*^{s} *rec*A1 recipient to *rif*^{d} occurs at the much lower frequency of 2×10^{-6}.

Since a portion of the chromosome of a specialized transducing phage is bacterial DNA, in Rec$^+$ cells transduction can generally

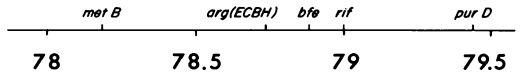

FIG. 1. *Genetic map of the bfe region of the chromosome. The order and relative distances of the metB, arg(ECBH) and purD loci are from Taylor and Trotter (33). The map position of rif*^{d} *relative to argH-2 and purD is from Kirschbaum (Ph.D. thesis) and the position of bfe is from the data presented in Table 2. Bfe is 88% co-transducible with rif*^{d}.

TABLE 3. *Induction of λcI857s7 integrated at bfe gives low-frequency transducing lysates for argE and rif*^{d} [a]

Recipient	Frequency of transduction for	
	Rif^d	*Arg*$^+$
rif^s (H102)	7.5×10^{-5}	
rif^s *recA* (H105)	2.0×10^{-6}	
rif^s *argE* (H113)	6.4×10^{-5}	3.6×10^{-4}

[a] A highly concentrated phage lysate prepared from XT113 was analyzed for its ability to transduce bacterial markers (*argE*, *rif*^{d}) on either side of the prophage integrated at *bfe*. The transductions were performed as described in Materials and Methods, and the values represent the number of transductants per PFU in the low-frequency transducing lysate. The relevant genotypes of the recipients used in each transduction are given, together with the actual strain designation given in parenthesis. The spontaneous background of Arg$^+$ revertants was at a frequency of 4×10^{-8}, and the frequency of spontaneous mutation to rifampin resistance was 2×10^{-8}.

occur by either of two types of events: (i) recombination between the bacterial segment of the phage chromosome and the homologous region of the bacterial chromosome, or (ii) insertion of the transducing phage at the normal *att*λ site on the bacterial chromosome. In this case, recombination directed by the phage-coded *int* function (17) occurs between the *att*λ site and the corresponding attachment site on the prophage. The types of *rif*^{d} transductants which could arise by these various recombination schemes are diagramed in Fig. 2. In cells carrying the *recA1* allele, the frequency of recombination between homologous regions of DNA is reduced by approximately 10^4-fold (12, 35). Therefore, transduction to *rif*^{d} in a Rec$^-$ recipient is most likely to occur by integration of the phage at the *att*λ locus. Clearly, only those λd*rif*^{d} phage integrating the *att*λ site which carry an intact *rif*^{d} gene will confer rifampin resistance.

Isolation of high-frequency transducing lysates for rif^d. From the above discussion, it is obvious that selecting for *rif*^{d} transducing phage in Rec$^-$ cells should greatly favor the isolation of phage carrying the *rif*^{d} gene in complete form. For this reason, we focused our attention on the Rec$^-$ *rif*^{d} transductants.

These transductants were isolated at a frequency approximately 100-fold higher than spontaneous *rif*^{r} mutants. Moreover, heat-pulse curing treatment of these transductants results in a loss of rifampin resistance, as expected if they are lysogenic for a heat-inducible *rif*^{d} transducing phage. In addition, heat induction of these lysogens produces high-titer phage λ

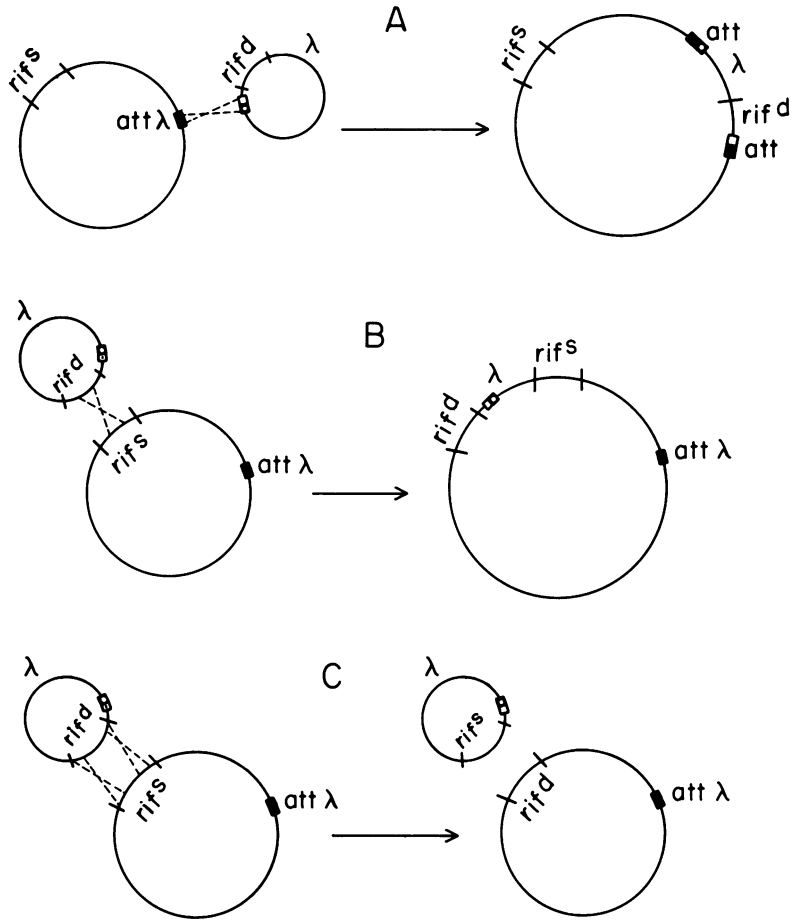

FIG. 2. A *presents the normal insertion of the λrif^d phage into the chromosomal λ attachment site. This event requires the phage int function which promotes specific recombination between the attλ site and the corresponding region on the phage. If the transducing phage carries the complete rif^d gene, then integration by this mechanism gives rise to a rif^s/rif^d heterodiploid which is rifampin resistant and has λ immunity. If the phage instead carries only a fragment of the rif^d gene, the lysogen formed by this mechanism would not be rifampin resistant.* B *diagrams the integration of the λrif^d phage into the rif region of the bacterial chromosome. This event requires a single crossover between the regions of homology. If the phage carries the intact rif^d gene, then insertions of this type produce a rif^s/rif^d heterodiploid which is rifampin resistant and λ immune. If the phage carries only a fragment of the rif^d gene, then transduction to rif^d would not occur except in those cases in which the gene fragment actually contained the rif^d point mutation. In this case, insertion of the phage by a mechanism involving a single reciprocal crossover between this rif^d gene fragment and the rif^s region of the bacterial chromosome would give rise to a functional rif^d gene and an incomplete rif^s gene.* C *diagrams how the recipient cell could become rif^d as a consequence of infection by the transducing phage, without yielding a stable rif^s/rif^d lysogen. A double reciprocal crossover between the homologous regions of the transducing phage and the bacterial chromosome yields a rif^d bacterial recombinant which could then segregate the phage. Such a rif^d cell would not have λ immunity and could not yield a high frequency transducing lysate upon induction.*

lysates which transduce *rif^d* at frequencies as high as 5×10^{-2}. Table 4 shows that for each of the *rif^d* phage isolated, transduction to *rif^d* is approximately 100-fold more efficient in Rec$^+$ strains than in strains which are Rec$^-$

These high-frequency transducing lysates were prepared from transductants which carried a wild-type helper phage in addition to the *rif^d* transducing phage. These lysates therefore contain a mixture of wild-type and transducing phage particles. The fact that the frequency of transduction is significantly lower in Rec$^-$ cells suggests that the *rif^d* phage cannot integrate efficiently by the *int*-promoted mechanism into

TABLE 4. *High frequency of transduction to rif^d* [a]

Phage no.	Titer of lysate (PFU/ml)	Frequency of transduction to *rif^d* in	
		Rif^s Rec$^+$	*Rif^s Rec$^-$*
5	2.9×10^9	5.4×10^{-2}	2.3×10^{-4}
12	1.1×10^{10}	1.3×10^{-2}	1.2×10^{-4}
18	2.5×10^{10}	3.0×10^{-2}	3.5×10^{-4}
25	2.0×10^9	8.7×10^{-4}	1.4×10^{-6}
28	3.3×10^9	1.0×10^{-4}	6.6×10^{-7}
29	7.7×10^9	4.6×10^{-4}	1.5×10^{-6}

[a] Lysates for each of the indicated phages were prepared by heat induction of double lysogens of the type H105 (λcI857s7, λcI857sd*rif^d*). The Rif^s Rec$^+$ recipient was strain H102, and the otherwise isogenic Rif^s Rec$^-$ recipient was strain H105. Transduction frequencies are expressed as the number of transductants per PFU in the lysate.

the normal *att*λ region of the chromosome, even in the presence of a wild-type phage supplying the *int* function. A likely explanation is that a portion of the attachment region of the transducing phage is derived from the *bfe* locus, a site normally recognized with only low efficiency by *int*. Therefore, the integration of such a phage into *att*λ by the *int*-promoted mechanism might be extremely inefficient. The preferred site of integration is most likely the homologous *rif* region where insertion can occur by *rec*-promoted recombination.

λdrif^d phage carries an intact rif^d gene. It is important to determine the site of integration of the λd*rif^d* phages in those lysogens producing high-frequency transducing lysates. In those Rec$^-$, rifampin-resistant transductants where the transducing phage has integrated in *att*λ, one can unambiguously conclude that the phage carries a complete *rif^d* gene. Mapping experiments were performed based on the following argument: if these *rif^d* transductants carry the transducing phage *att*λ, then one would expect *rif^d* to be linked to the nearby *gal* genes. Such linkage would not be found if the *rif^d* phage were integrated in the *rif* region of the chromosome.

Figure 3 shows the origin and direction of transfer of the chromosome from the Hfr strain H105 (Hfr KL16, *thi*, *rec*A1) used for the isolation of the λd*rif^d* lysogens. Each of the lysogens described in Table 4 was used as a donor in matings with an F$^-$ *rif^s gal strA* recipient in which Gal$^+$ Str^R recombinants were selected and scored for Rif^d. Table 5 shows that in the majority of lysogens tested, approximately 80% of the Gal$^+$ recombinants were also Rif^d, whereas a control donor carrying a spontaneous *rif^r* mutation gave only Rif^s Gal$^+$ Str^R recombinants.

Two of the *rif^d* lysogens failed to yield Gal$^+$ Str^R recombinants which were also Rif^d. This result suggests that the *rif^d* transducing phage in these lysogens is not located at the *att*λ site. Lysates prepared from these strains nonetheless

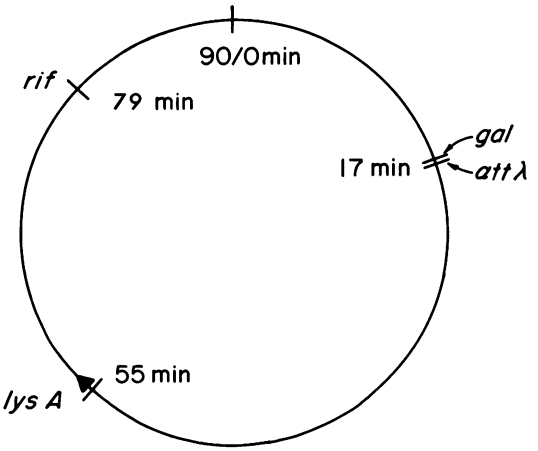

FIG. 3. *Origin and direction of transfer of the bacterial chromosome in strain H105. Strain H105 is HfrKL16, with the origin and direction of transfer indicated by the arrowhead (26). The relative positions of the att*λ*, gal and rif loci on the standard E. coli genetic map are as indicated.*

TABLE 5. *Site of prophage insertion, determining the linkage of rif^d with gal in the λdrif^d lysogens by Hfr crosses*[a]

Donor	No. Gal$^+$ recombinants scored	No. which are Rif^d	Rif^d Gal$^+$ (%)
λd*rif^d* lysogen			
No. 5	110	88	80
12	110	0	0
18	110	88	80
25	110	85	77
28	110	0	0
29	110	85	77
Control	110	0	0

[a] Hfr donor strains are derivatives of H105 (HfrKL16, *thi*, *rec*A1) which are double lysogens carrying both λcI857s7 helper and each of various λcI857s7d*rif^d* transducing phages. The donor for the control mating is H117 (Hfr KL16 *thi*, *rif^r*, *rec*A1, (λcI857s7). The recipient for these crosses was a wild-type lambda lysogen of X217 (F$^-$ *gal*-2 *str*A). Matings and selection of Gal$^+$ Str^r recombinants were as described in Materials and Methods. The recombinants from each cross were gridded onto the same selective medium and were scored for Rif^d by replica plating on TYE plates with and without 100 μg of rifampin per ml. The estimated frequency of transfer was on the order of 10^{-3} to 10^{-4} Gal$^+$ Str^r recombinants per Hfr. The spontaneous rate of reversion to Gal$^+$ of X217 (λ$^+$) was less than 10^{-8}.

transduce *rif*^{d} at frequencies comparable to the *att*λ lysogens (Table 4). Analysis of the Rif^d transductants of strain H105 (*rif*^{s} *recA1*) obtained with these high-frequency transducing lysates has shown that approximately 85% of the cells lysogenic for a given λd*rif*^{d} prophage carry the λd*rif*^{d} phage at the *att*λ site. For those lysogens in which the prophage is not at *att*λ, it is unlikely that the λd*rif*^{d} has integrated by recombination into the homologous region of the bacterial chromosome because the recipient cells are Rec$^-$ and the generalized recombination function of the phage lambda (*red*) cannot promote recombination of this type (18). Perhaps integration occurs by *int*-promoted recombination between the hybrid attachment site carried by the phage and the *bfe* locus.

A direct demonstration that the λd*rif*^{d} phage carries at least the *rif*^{d} gene in intact form is shown by the fact that substantial amounts of both rifampin-resistant and rifampin-sensitive RNA polymerase activity are present in the transductants. Figure 4 compares the amount of resistant RNA polymerase activity in extracts from the uninduced *rif*^{s}/λd*rif*^{d}18 lysogen and its otherwise isogenic *rif*^{s} counterpart. At the minimum concentration of rifampin which completely inhibits the RNA polymerase in the *rif*^{s} extract, approximately half of the enzyme activity in the *rif*^{s}/*rif*^{d} transductant is resistant. Enzyme prepared from a *rif*^{d} haploid is completely resistant under these same conditions (Kirschbaum, Ph.D thesis). This high level of *rif*^{d} gene expression in the uninduced lysogen suggests that the *rif*^{d} gene is not under phage lambda control, and that the λd*rif*^{d}18 phage probably carries the *rif* promoter as well.

These findings confirm and extend the genetic argument that the gene for the β subunit of RNA polymerase is carried by the phage in an intact form, and that this gene product is active not only in vivo but also in vitro.

Other properties of the λd*rif*^{d} transducing phages. The *rif*^{d} phage are unable to form plaques. Infection of *rif*^{s} *recA* cells at low multiplicity with λd*rif*^{d} lysates in the absence of added helper phage produces single lysogens which are rifampin resistant (the transducing efficiency is not helper dependent). Although heat-pulse curing treatment of these lysogens causes a loss of the Rif^d phenotype, heat induction fails to produce detectable phage after chloroform treatment of the induced cells. In contrast, heat induction of these single lysogens immediately after superinfection with wild-type helper phage produces a high-titer plaque-forming lysate which transduces *rif*^{d} at a high frequency. In addition, centrifugation of a λd*rif*^{d}18 high-frequency transducing lysate to equilibrium in a CsCl density gradient yields two well-resolved phage bands. The lighter band contains the wild-type phage, whereas the heavier band contains the transducing phage and accounts for approximately one third of the total phage particles (as determined by absorbance at 260 nm.). This purified transducing phage is defective in plaque-forming ability.

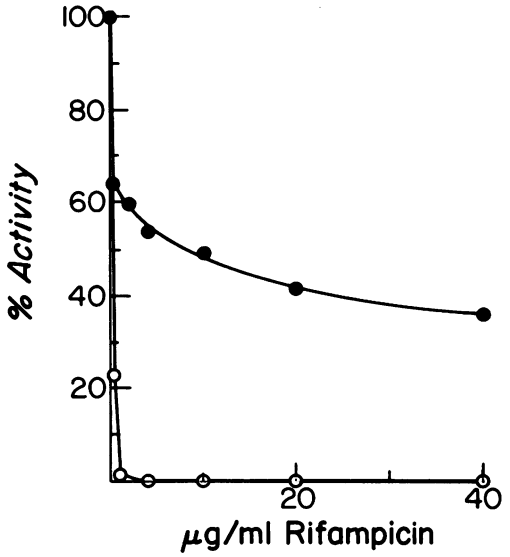

FIG. 4. *Effect of rifampin on RNA polymerase activity in vitro. The upper curve represents the RNA polymerase activity in an extract prepared from the uninduced double lysogen H105 (λcI857s7, λc857s7drif^{d}18). The lower curve represents the control extract prepared from the otherwise isogenic rif^s lysogen, H105 (λc1857s7). The specific activities of the partially purified enzyme preparations from the λdrif^{d}18 and wild-type lysogens were 3.2 × 10^4 and 3.3 × 10^4 counts per min per mg of protein, respectively.*

Therefore, the bacterial DNA carried by these phage must have replaced vital phage genes. The fact that stable *rif*^{d} single lysogens can be isolated in Rec$^-$ cells suggests that the phage must have integrated by an *int*-promoted insertion and that at least this gene must not have been affected by the substitution. Assuming that the defective *rif*^{d} phage arose according to the Campbell model (9), it is likely that these phage are structurally similar to the λd*gal* class of transducing phage, in which a portion of the DNA in the left arm of the phage has been replaced by the *gal* genes (1). Evidence for such a structure has been suggested by preliminary heteroduplex mapping analysis of the λd*rif*^{d}18 phage (J. Wolfson, personal communication).

This phage also contains more DNA than the wild-type particles. As mentioned above, the λd*rif*^{d}18 phage bands at significantly higher density than wild-type in a CsCl density gradi-

ent. Measurements of the contour length of 30 λ*drif*^{d}18 DNA molecules in the electron microscope have established a molecular weight of $32.6 \pm 1.5 \times 10^6$, by using φX174 circular double-stranded DNA as a standard (J. Wolfson, personal communication). The λ*drif*^{d}18 phage therefore contains approximately 6% more DNA than wild-type λ (13).

DISCUSSION

The isolation of *rif* transducing phages provides a method for obtaining phages which carry at least the β subunit gene of *E. coli* RNA polymerase. Due to a lack of mutations in the genes for the other polymerase subunits, it is not known whether these genes form a single operon with the gene for the β subunit. Therefore, the existence of a *rif* transducing phage carrying additional subunit genes would provide direct evidence that these genes do, in fact, belong to the same operon. It may be possible to determine whether these *rif* phages carry other subunit genes as well, by analyzing the proteins synthesized under the direction of the transducing phage DNA in a DNA-dependent protein synthesizing system of the type developed by Zubay et al. (37) for the synthesis of specific bacterial proteins.

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