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Isolation and Characterization of ϕ80 Transducing Bacteriophage for a Ribonucleic Acid Polymerase Gene

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A new method for isolating specialized transducing phages is described. It was used to isolate a group of ϕ80 transducing phages which carry various bacterial markers from the *metB* region of the *Escherichia coli* chromosome. Some of the phages selected for transduction of the *supA36* marker were also shown to carry *rif*, a locus known to specify the β subunit of ribonucleic acid polymerase. Expression of the prophage *rif*^{r} gene in lysogens was demonstrated by its ability to confer rifampin resistance on part of the cellular ribonucleic acid polymerase pool.

Specialized transducing phages have been isolated for a number of different groups of genes derived from the *Escherichia coli* chromosome. Such phages have been very useful for the genetic study of the incorporated genes, and have provided a source of deoxyribonucleic acid (DNA) for the study of bacterial gene expression in vitro. Ordinarily, loci for which specialized λ or ϕ80 transducing phages can be isolated are limited to those which map near the prophage insertion sites for these phages (*att*λ or *att80*) on the *E. coli* chromosome. However, methods have been described recently which permit the isolation of transducing phages for almost any region of the bacterial chromosome. The genes to be incorporated can be transposed so that they lie close to *att*λ (9) or *att80* (6), or the λ prophage can be inserted near the required genes (14). This paper describes a new method for the isolation of transducing phages which we have used to isolate a group of ϕ80 phages that carry a number of different genes for the *metB* region of the chromosome (Fig. 1). Some of these phages carry *rif*, a locus known to specify the β subunit ribonucleic acid (RNA) polymerase (7).

RNA polymerase (EC 2.7.7.6) is a complex structure of central importance to the cell. Its genetic organization is little understood. The holoenzyme consists of four different subunit species (3) of which only one, β, has been positively identified with a genetic locus on the *E. coli* chromosome. This identification was made possible by the discovery that mutants resistant to rifampin and streptolydigin, drugs which inhibit RNA polymerase, have an altered β subunit, and that such mutations (*rif*^{r}, *stl*^{r}) can be mapped at a discrete locus (*rif*) in the *metB* region of the chromosome (7). Are genes for any of the other polymerase subunits situated near the *rif* locus, perhaps forming a coordinately transcribed unit? Is the expression of any or all of such cistrons regulated? The isolation of specialized transducing phages for the *rif* region may provide a valuable tool for the study of these problems. We may be able to use DNA isolated from the ϕ80d*rif* phages to direct the synthesis of polymerase subunits in vitro, to determine which subunits are made and whether control factors are involved in this expression.

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MATERIALS AND METHODS

Bacteria. All bacterial strains were from the collection of J. Beckwith. Table 1 shows the genotypes of strains mentioned in the text.

Use of bacteriophages. Lysates of phage ϕ80 and its derivatives were prepared and used for specialized transduction as described by Signer (15). Prototrophic transductants of auxotrophic strains were selected by plating on suitably supplemented M63 agar plates (13), which lacked the relevent auxotrophic requirement. All recipient strains were ϕ80 lysogens. Purified high-titer lysates were prepared by cesium chloride block gradient centrifugation (18).

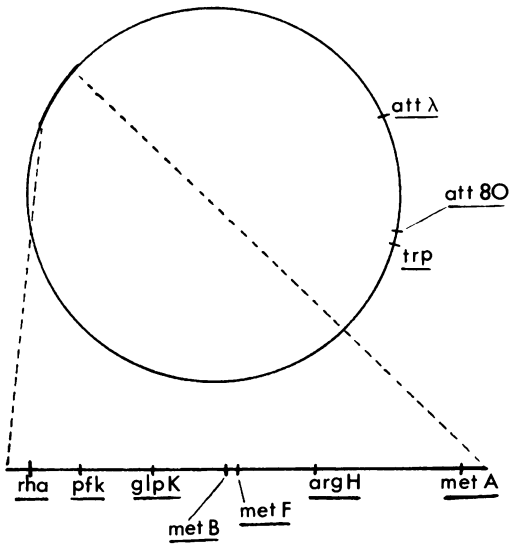

FIG. 1. *Map of the E. coli chromosome showing relative positions of some of the loci mentioned in the text. Taken from Taylor and Trotter (17).*

TABLE 1. *E. coli strains used*[a]

Strain	Relevant genetic markers	φ80 Prophages
X7187	*metB*⁻	
XI 7564	*metB*⁻	φ80 · φ80d*metB*⁺
XI 7566	*supA*36 *ins*⁺₅₇₆ *trpA*₃₆ *glyS*₁₀ *rif*ˢ	φ80 · φ80d*metB*⁺
XI 7571	*supA*36 *ins*⁺₅₇₆ *trpA*36 *glyS*₁₀ *rif*ʳ − X71	φ80 · φ80d*metB*⁺
W7008	*trpA*36 *argH*⁻ *rif*ˢ	φ80
W9005	*trpA*36 *rif*ʳ-5W	
IR.O	*metB lac* Y14 *recA trpA*36 *rif*ˢ	
IR.O·1	*metB lac* Y14 *recA trpA*36 *rif*ᵒ −.A/F'14 *metB*⁺*supU*	

[a] Mutations: *supA*36, missense suppressor; *ins*₅₇₆, suppressor which renders *supA*36 haploid strains viable; *trpA*36, missense mutation which is suppressed by *supA*36; *glyS*₁₀, mutation in glycyl-tRNA synthetase; (the preceding mutations are described in Carbon, Squires, and Hill [19], and were furnished by C. Hill) *rif*ˢ, wild-type *rif* locus; *rif*ʳ, mutation conferring resistance to rifampin; *lac*Y14, amber mutation in *lac*Y; F'14, episome which covers the *metB* region but not *rif*; *supU*, amber suppressor; *rif*ᵒ, amber mutation in *rif* (2).

Co-transduction frequencies for bacterial markers were determined by the use of P1 *vir*, a mutant of phage P1 isolated by B. Wolf, which cannot lysogenize. The P1 transductions were performed according to Signer (15) except that 0.2 ml of 1.0 M sodium citrate was added to each plate.

Assays for RNA polymerase activity. Stationary-phase bacterial cultures were extracted and assayed as previously described (2). Rifampin resistance of the in vitro activity was tested in the presence of 10 μg of rifampin per ml (Lepetit, Milan), unless otherwise indicated. Crude supernatant fluids were assayed after centrifugation at 100,000 × *g* for 90 min. Final extracts were partially purified and completely dependent on added DNA for activity.

Media. All media used were as described by Gottesman and Beckwith (6). Rifampin was added to agar plates to 50 μg/ml when required.

Abbreviations. Designations for genetic loci are taken from Taylor and Trotter, except where otherwise noted.

RESULTS

Isolation technique for new transducing phages. The method we have developed for the isolation of transducing phages for loci in the *metB* region is illustrated in Fig. 2. Prophage insertion by the Campbell model (5) occurs by reciprocal recombination between the host chromosome and the circularized phage chromosome. In the case of wild-type phage insertion, recombination is between the host and phage attachment sites. For a transducing phage, recombination may occur between host loci carried by the phage and homologous loci

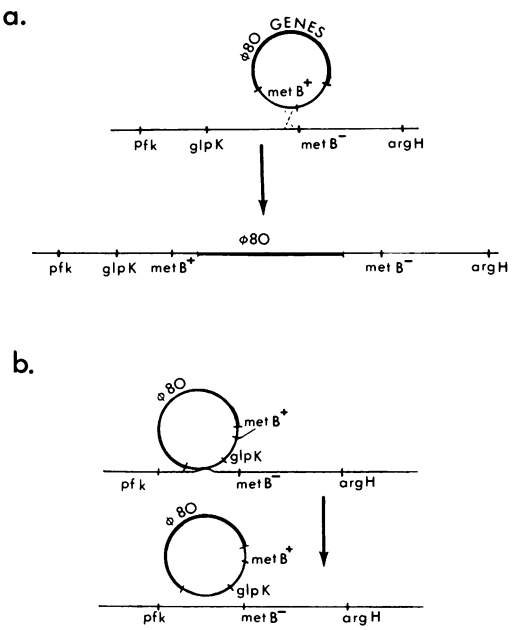

FIG. 2. (*a*), *Reciprocal recombination between φ80dmetB and the metB locus of the host inserts the defective prophage into the host chromosome and generates a duplication of the metB locus. Insertion to the right of the metB⁻ mutation is illustrated. Insertion to the left would reverse the sequence of metB⁺ and metB⁻ in the product.* (*b*), *Nonhomologous excision generates a phage carrying the glpK locus.*

on the host chromosome (16). We have taken advantage of this second type of insertion to introduce a φ80d*metB* transducing phage, fortuitously isolated by B. Konrad (Ph.D. thesis, Harvard University), into the *metB* locus, as shown in Fig. 2a. Induction of this inserted prophage can now generate transducing phage for loci near *metB* by rare nonhomologous excision events of the sort shown in Fig. 2b. If a wild-type helper phage is also present in the cell, these new defective transducing phages appear in the lysate and can be isolated.

Isolation of a φ80dmetB lysogen with the prophage inserted at metB. A *metB*$^-$ strain, (X7187) was transduced to *metB*$^+$ by a lysate of φ80d*metB*$^+$ and φ80 phage, selecting for the methionine independent growth character of the transductants.

To isolate an insertion of the type shown in Fig. 2 from among the transductants, three transductants were purified and tested to determine whether they yielded φ80 LFT transducing lysates for the *glp*K locus (Fig. 2b). One transductant (XI7564) was found to have this capacity (Table 2). Furthermore, lysates of the generalized transducing phage P1*vir* made on X17564 co-transduced the prophage φ80d*metB* with *glp*K, and also showed decreased co-transduction of *glp*K and *arg*H (7% co-transduction, compared to 17% co-transduction with P1*vir* lysates grown on X7187). These results are compatible with the new linkage relationships anticipated from the insertion of φ80d*met*B at the *met*B locus. The wild-type φ80 helper prophage which was also present in strain XI7564 could be eliminated by crossing a deletion of *att*80 into XI7564. This prophage must, therefore, be inserted at *att*80, rather than at *met*B with the φ80d*met*B prophage.

Isolation of transducing phages for regions adjacent to metB. Phage φ80 lysates made from XI7564 were LFT transducing lysates for other loci near *met*B, as well as for *glp*K (Table 2). In general, the frequency of transductants decreased with increasing distance from *met*B, the transduction for either *sup*A36 or *met*A being the exception.

These transductants yielded HFT lysates which could transduce the selected markers at high frequency. We have tested the transducing phages isolated in this way for various markers in the *met*B region (Table 3). Most of these phage appear to carry the whole segment of the chromosome which lies between *met*B and the selected marker. We propose that these arose by the general mechanism illustrated in Fig. 2b. Phages carrying *arg*H, *met*A, and *sup*A36 were found at lower frequencies and never carried all of the intervening loci. These markers are too distant from *met*B for inclusion of the entire segment of the host chromosome in a phage to be possible, so a part of this segment is necessarily deleted in these phages.

TABLE 2. *Frequencies of transducing phage in lysates from XI7564 and XI7571*[a]

Marker	Frequency in LFT lysate
glpK	10^{-6}
pfk	10^{-7}
rha	2.5×10^{-9}
argH	5×10^{-10}
supA36	10^{-11}
metA	1.7×10^{-10}

[a] Frequencies are expressed as the number of transductants per plaque-forming unit of helper phage. The *sup*A36 transduction was done with XI7571.

Isolation of transducing phage carrying rif. To permit its isolation as a transducing phage, a phage must carry a dominant bacterial marker that can be selected for in the host cell. Rifampin resistance is usually recessive to sensitivity (1). Thus, a φ80d*rif*^{r} phage will not ordinarily confer rifampin resistance on a φ80d*rif*^{r}/*rif*^{s} lysogen and cannot be selected directly. We have, therefore, selected transducing phages, which carried a dominant marker closely linked to *rif*, and screened for those which also carried the *rif* locus.

The available marker nearest to *rif* is the *sup*A36 missense suppressor gene (12) (Fig. 3). Forty transducing phages carrying *sup*A36 were isolated from lysates of a derivative of XI7564, XI7571 (*sup*A36 *trp*A36 *rif*^{r}X-71 [φ80d*met*B] [φ80]) (Table 2), by selecting for their ability to restore tryptophan-independent growth to the suppressible *trp*A mutant recipient strain W7008 (*trp*A36 *rif*^{s}). Those transductants which had received phage that carried the *rif*^{r}X-71 mutation as well as the closely linked *sup*A36 locus would remain sensitive to rifampin, however we anticipated that they would segregate φ80d*sup*A36*rif*^{r}X-71/*rif*^{r}-X71 homogenotes, formed by recombination between the *rif* alleles, at a high frequency. Thirteen of the 40 transductants were found to segregate clones of homogenotes resistant to rifampin at frequencies of 10^{-2} to 10^{-3} per viable cell per generation. These are several orders of magnitude higher than the corresponding frequency of 10^{-8} for spontaneous mutation to rifampin resistance in strain W7008. Moreover, lysates made from these 13 transductants were found to transduce this capacity to form rifampin-resistant homogenotes together with the *sup*A36 locus. We conclude that these 13 transductants are lyso-

TABLE 3. *Loci carried by transducing phage isolated from XI7564*[a]

Selected for	Scored for							
	rha	*pfk*	*glpK*	*metB*	*metF*	*argH*	*purD*	*metA*
metB	−	−	−	+				
glpK	−	−	+	+				
pfk	−	+	+	+				
rha	+	+	+	+				
argH				19/50	5/12	12/12	0/12	0/12
metA				1/12	0/4	0/4	0/4	4/4

[a] The + indicates the locus was always found, − indicates the locus was never found among the one or more phage tested. Otherwise, the denominator is the number of phage tested, and the numerator is the number of phage which were found to carry the indicated locus. The phage isolated from XI7571 which carry *sup*A36 do not include any of these loci.

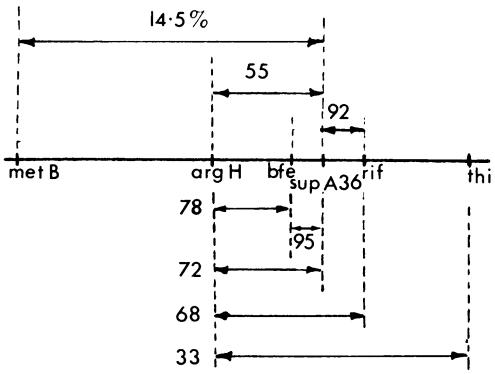

FIG. 3. *Partial map of the rif region constructed from transductions with P1vir. The order of loci was derived from three point crosses. Numbers are percent co-transduction. The bfe locus determines sensitivity to phage BF23 (4). Our mapping data for this region are in good agreement with those of Jasper et al. (10) and Orias et al. (12).*

genic for transducing phage that carry the *rif*[r]X-71 mutation as well as *sup*A36.

Rifampin-resistant polymerase in ϕ80drif[r] lysogens. Although HFT lysates prepared from each of the 13 ϕ80d*sup*A36*rif*[r]X-71 lysogens transduce both *sup*A36 and *rif*[r]X-71 at a high frequency (10^{-3} transductants per plaque-forming unit in the lysate), they do not transduce *thi*, the closest known marker to *rif* on the side distal to *sup*A36 (Fig. 3). Thus, it is possible that these phage include only a fragment of the *rif* gene which contains the *rif*[r]X-71 mutant site. It is important, therefore, to determine whether they can direct the synthesis of an intact *rif*[r] gene product in the absence of recombination. This would indicate the whole structural gene is present on the phage.

Lysogens of five ϕ80d*rif*[r] phage in a recombination deficient, rifampin-sensitive strain (IR.0) were constructed and tested for the presence of RNA polymerase activity resistant to rifampin. Figure 4 shows that partially purified polymerase from the ϕ80d7-*rif*[r] lysogen has two components. One, accounting for about 80% of the total activity, is completely sensitive to rifampin, whereas the other shows the same resistant properties as extracts from the *rif*[r] haploid strain from which the transducing phage was derived. The four other ϕ80d*rif*[r] lysogens tested gave similar results, whereas a lysogen containing the ϕ80d$_s$*supA36* phage, which does not carry a *rif*[r] site, yielded polymerase which was completely sensitive to rifampin (Table 4).

Stationary phase cells of *rif*[s]/*rif*[r] heterodiploid strains normally contain equal proportions of rifampin-resistant and sensitive polymerase molecules (2). The low proportion of *rif*[r] enzyme in extracts of *rif*[s] (ϕ80d*rif*[r]-X71) lysogens is probably due to the relative instability of the *rif*[r]-X71 gene product in vitro rather than to a low rate of expression of the prophage *rif* gene. This instability can be demonstrated by comparing an extract of a *rif*[r]-X71 haploid strain (XI7571) with one from an isogenic *rif*[s] strain (XI7566). Crude extracts of these two strains show similar polymerase activities when assayed immediately after preparation. However, after the purification process, the *rif*[r]-X71 product has threefold less total activity than the *rif*[s] strain (Table 4). This suggests that some 70% of the *rif*[r]-X71 product is inactivated during the purification procedure. Thus, although the final yield of the prophage directed *rif*[r]-X71 product is low, the rate of expression of the gene is probably normal. This conclusion is supported by the properties of a recombinant phage, ϕ80d7*rif*[s], whose *rif*[r]-X71 site has been replaced by a *rif*[s] allele. The *rif* gene of this phage is expressed fully, since about half of the polymerase activity extracted from a ϕ80d7*rif*[s] lysogen derived from strain W9005, which carries a new *rif*[r] mutation at the chromosomal site, shows

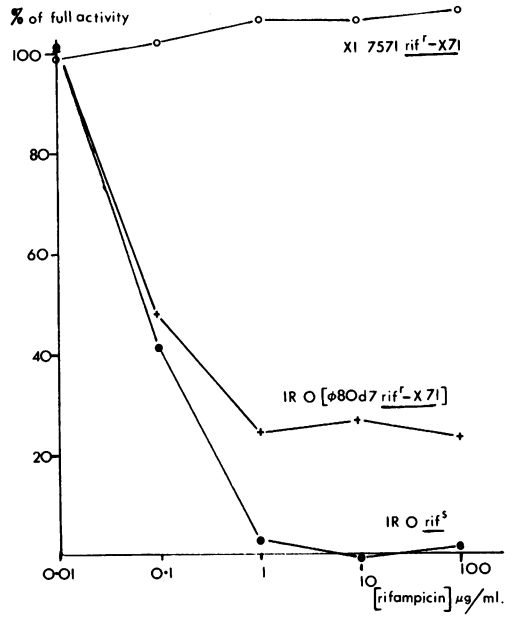

FIG. 4. *Response of partially purified RNA polymerase extracts to rifampin. Strain IRO (rif^s argG$^-$ metB$^-$ trpA36 str^r recA$^-$) was lysogenized by φ80d7rif^r-X71 supA36 by selecting for tryptophan independence. The phage derives its rif locus from the control strain XI7571 (rif^r-X71).*

TABLE 4. *Activity of RNA polymerase preparations*[a]

Strain used for polymerase extract	Activity in crude lysate (nmol of AMP per g of cells)	Total activity in partially purified extracts (nmol/g)	Rifampin resistant activity (%)
IR O *rif^s*		53	0
IR O *rif^s* (φ80d8supA36)		65	0
IR O *rif^s* (φ80d7*rif^r*)		44	21
IR O *rif^s* (φ80d18*rif^r*)		51	16
IR O *rif^s* (φ80d19*rif^r*)		55	19
IR O *rif^s* (φ80d33*rif^r*)		48	21
IR O *rif^s* (φ80d39*rif^r*)		44	18
X I 7571 *rif^r*	12	12	114
X I 7566 *rif^s*	13[b]	41[b]	0
IR O·1*rif^o*-A(φ80d18*rif^r*)	16	15	108
W900 *rif^r*-5W(φ80d7*rif^s*)		51	54

[a] Activities are expressed as nanomoles of AMP incorporated per gram (wet weight) of cells used in a 10-min assay.

[b] Crude polymerase preparations from *rif^s* and most *rif^r* strains show a threefold increase in total measurable polymerase activity on purification (2).

the prophage directed *rif^s* character (Table 4).

Reversal of the rif^o mutant phenotype by φ80d18rif^r. We have previously described the isolation of nonsense mutations which eliminate the expression of *rif^s* alleles (*rif^o* mutations) (2). In the absence of a second copy of the *rif* locus such mutants are entirely dependent for growth on the presence of the amber suppressor *supU* which is located on a resident F′ episome. We introduced φ80 d18*rif^r* into such a suppressed *rif^o*-amber strain, IRO·1. In contrast to the nonlysogenic strain, the lysogen could readily loose the F14 *supU* suppressor episome on acridine orange curing (8). It seems likely that *rif^o*-amber mutations eliminate the expression of the β-subunit cistron of RNA polymerase in su$^-$ cells (2). Thus, the resident φ80d18*rif^r* phage allows growth of these cells as it carries an intact β-cistron. As expected, the *rif^o*-amber (φ80d18*rif^r*) strain was resistant to rifampin, and yielded a polymerase extract which was entirely rifampin resistant (Table 4).

DISCUSSION

We have developed a new technique for isolating specialized transducing phages which can be applied to any marker that lies reasonably close to a locus for which a transducing phage already exists, and that can be selected. By sequential repetition of this technique, it should be possible to isolate sets of phages which cover large sections of the bacterial chromosome relatively easily. The scope of this method can be increased by screening new phages for the presence of nonselective markers which are closely linked to the selected locus.

We have used the new selection technique to isolate phages which carry the missense suppressor gene *supA36*. In vivo some of these phages direct the synthesis of a functional β subunit of RNA polymerase. In one case the levels of phage directed product indicate that the prophage located β gene is expressed at a rate similar to that of a chromosomally located allele. This suggests that the prophage gene remains under the control of its normal promoter.

The fact that a φ80d*rif^r* prophage can complement nonsense mutations of the *rif^o* class supports our previous conclusion that *rif^o* mutations eliminate the synthesis of the β subunit of RNA polymerase from chromosomally located *rif* genes (2).

Studies on apparent polarity effects of *rif^o* mutations have suggested that at least the β and β′ genes form a transcriptional unit in *E. coli* (Hayward, Austin, and Scaife, manuscript in preparation). Thus, polymerase genes other than β may be present on the φ80d*rif^r* phages. We are currently studying the in vitro products directed by φ80d*rif^r* phage DNA in a coupled transcription-translations system in an attempt to answer this question. We hope that

this system will also prove useful for studies on the control of expression of RNA polymerase genes.

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