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Formation of a λ(Tn*10*) *tyrR*$^+$ Specialized Transducing Bacteriophage from *Escherichia coli* K-12

CHRISTOPHER S. COBBETT AND J. PITTARD*

Department of Microbiology, University of Melbourne, Parkville, Victoria, Australia

The transposon Tn*10*, coding for resistance to tetracycline, was inserted close to the *tyrR*$^+$ gene at min 28 on the *Escherichia coli* chromosome. The homology between this transposon and a λ(Tn*10*) phage was employed to direct integration of λ close to *tyrR*$^+$ with subsequent isolation of a λ(Tn*10*) *tyrR*$^+$ transducing phage. Results of restriction endonuclease analysis of the transducing phage are presented.

Campbell (4) has described the formation of specialized λ *bio*$^+$ and λ *gal*$^+$ transducing phage from a wild-type lysogen. The generation of specialized phage in vivo which transduce loci distant from the λ integration site, *att*λ, can be achieved by transposition of the particular locus to a position adjacent to *att*λ, or by integration of the prophage at an unusual site close to the locus in question. A variety of general methods for the isolation of novel transducing phage by these means have been described (2, 5, 13, 14, 25, 26).

In this paper, we describe an additional general method involving the directed integration of λ at an unusual site. This was achieved by homologous recombination between a transposon, Tn*10*, inserted close to the particular gene, and a λ(Tn*10*) phage (16). The gene for which we have applied this method is *tyrR*$^+$, a regulatory gene of considerable interest involved in the regulation of the biosynthesis of the aromatic amino acids in *Escherichia coli* (3, 9, 28).

MATERIALS AND METHODS

Organisms. Bacterial strains, all derivatives of *E. coli* K-12, and λ bacteriophage used in this work are listed in Table 1. Strains NK5012 and λNK55 were obtained from P. Reeves. Abbreviations of bacterial genotypes are those used by Bachmann and Low (1).

Media. The minimal medium used was half-strength 56 (56/2), described by Monod et al. (23), supplemented with glucose (0.2%), thiamine, and required amino acids. Nutrient media used were Luria broth (22) and nutrient agar (Oxoid). The *trkE* locus, a locus involved in potassium uptake, was screened on a medium deficient in potassium ions, K0.1 (11), and the *tyrR* locus was screened on minimal medium containing 10^{-4} M L-3-fluorotyrosine (Cambrian) as described previously (3). *tyrR* strains are able to grow on this medium. Tetracycline was used at a concentration of 20 μg/ml in nutrient medium and 5 μg/ml in minimal medium.

Transposition of transposon Tn*10*. Transpositions of Tn*10* to the chromosome of strain W3110 were obtained by the method of Kleckner et al. (16).

P1 transduction. The method used for transduction, using phage P1 *kc*, was that described previously (3).

Selection for the *supE* locus. The amber suppressor locus was introduced into strains JP2801 and JP2938 by P1 transduction selecting for His$^+$ and His$^+$ Trp$^+$ transductants, respectively. Transductants were then screened for their ability to produce phage λ on induction.

Selection of λ lysogens. λNK55 lysogens of JP2769 were selected as follows. An exponential culture of JP2769 was infected with λNK55 at a multiplicity of infection of 30 phage per cell and allowed to stand at 32°C for 30 min. Integration of λNK55 into the chromosome of JP2769 would be expected to occur at a low frequency. These rare lysogens were first enriched for by diluting the above mixture 1:10 in fresh broth to which had been added λ *cI* at a concentration of 10^8 plaque-forming units (PFU) per ml. Then, after 30 min at 32°C, portions were spread on nutrient plates seeded with 10^7 PFU of λ *cI* per plate and incubated overnight at 32°C. Growth was streaked for single colonies on nutrient plates at 32°C, and these were tested for λ lysogeny by cross-streaking against λ *cI* and by determining their ability to produce phage on induction.

Because λ cI can lysogenize in the presence of a resident prophage, two types of lysogens may be obtained: a single lysogen bearing only the λNK55 prophage, or a double lysogen bearing both the λNK55 and λ *cI* prophages. The following scheme was used to distinguish these two types. λNK55 has a temperature-sensitive repressor and requires the presence of an amber suppressor mutation for expression of the *O* gene and phage multiplication. Strain JP2769 is *sup*$^+$ (i.e., is unable to suppress amber mutations) and, if lysogenized by λNK55 alone, would not be lysed on induction at 42°C; however, a double lysogen with both λ *cI* and λNK55 prophages would be temperature sensitive and would produce viable phage on induction.

Propagation of λ phage. Thermally induced lysates and plate lysates were prepared by the methods of Miller (22).

λ Transductions. Cells grown overnight in Luria broth plus 0.4% maltose were infected at a multiplicity of 0.1 to 0.5 phage per cell in the presence of 10 mM

TABLE 1. *Description of strains of E. coli K-12 and bacteriophage λ used*[a]

Strain designation	Relevant characteristics	Source or reference
E. coli K-12		
JP2144	*tyrR366 his-29*(Am) *trpA9605*(Am) *ilv-1*	(3)
JP2152	*tyrR366 his-29*(Am) *trkE142*(Am) Δ(*kdpABC*)5	From JP2144 via several intermediate strains
JP2769	*his-29*(Am) *ilv-1 zci-2*::Tn*10*	From JP2144 by P1 *kc* transduction
JP2801	*his-29*(Am) *ilv-1 zci-2*::Tn*10* (λNK55)	λNK55 lysogen of JP2769
JP2802	As for JP2801, but *supE*	From JP2801 by P1 *kc* transduction
JP2803	As for JP2769, but with KLF29	From JP2769 by conjugation
JP2804	As for JP2801, but with KLF29	From JP2801 by conjugation
JP2861	Phage P2 lysogen of NK5012	
JP2862	As for NK5012, but *recA56 srl-1300*::Tn*10*	From NK5012 by P1 *kc* transduction
JP2864	*his-4 aroD6 proA2 mal-396 rpsL739* Sup$^+$	A spontaneous streptomycin-resistant, λ *vir*-resistant derivative of AB1360 (24)
JP2938	λ Spi (Tn*10*) *tyrR*$^+$ lysogen of JP2144	
JP2939	As for JP2938, but *supE*	From JP2938 by P1 *kc* transduction
NK5012	*thr leu supE*	(16)
W3110	Prototroph, Sup$^-$	(3)
Phage		
λNK55	*b*221 *c*I857 *c*III167::Tn*10* *O*29(Am)	(16)
λ Spi (Tn*10*) *tyrR*$^+$	*b*221 *c*I857 *c*III167::Tn*10* *O*29(Am)	From strain JP2939
λ Spi *chi* (Tn*10*) *tyrR*$^+$	Spontaneous, large plaque-forming derivative of λ Spi (Tn*10*) *tyrR*$^+$	

[a] The nomenclature of bacterial genotypes is that followed by Bachmann and Low (1). (Am) indicates an amber mutation; the position of the chromosomal insertion Tn*10*, a transposon coding for resistance to tetracycline, is given according to the notation described by Kleckner et al. (17). KLF29 is an F′*his* episome. Strain JP2864 can suppress the *O*(Am) mutation of λNK55; however, the particular suppressor locus has not been identified. The genetic designations of phage λ derivatives are those followed by Echols and Murialdo (8). Spi$^-$ indicates the ability of the λ derivative to form plaques on a strain lysogenic for phage P2, and *chi* is a mutation allowing the formation of large plaques by the λ Spi derivative (see text).

$CaCl_2$–$MgCl_2$ and allowed to stand at 32°C for 50 min to allow the expression of resistance to tetracycline. Portions of appropriate dilutions were then plated on selective media. When λ *c*I857 helper phage were used to increase the frequency of lysogeny, they were present at a multiplicity of infection of 1 to 5 phage per cell. When transductants were selected, the transductant colonies proved to be heterogeneous populations of lysogens and nonlysogens. Lysogenic cells were selected from these colonies by streaking the transductants on nutrient plates seeded with λ *c*I.

Zygotic induction. The site of prophage integration in the chromosome was confirmed by zygotic induction. Strains JP2803 and JP2804 were mated with JP2864. The mating was performed at 34°C, and samples were taken at 30-min intervals and plated on media selecting for His$^+$, Aro$^+$, and tetracycline-resistant transconjugants. Streptomycin (400 μg/ml) was used as a contraselection against the donor, and samples were also assayed for infective centers by using NK5012 as the plaquing strain. The recipient, JP2864, has an amber suppressor mutation allowing lysis to occur after zygotic induction and is *malT* (resistant to λ *vir*) to prevent infection by free phage particles during the mating.

Isolation of phage λ DNA. Phage were precipitated from 100 ml of a large scale lysate by the addition of 20 ml of 5 M NaCl and 40 ml of 40% polyethylene glycol. After standing overnight at 4°C, the precipitate was collected by centrifugation and resuspended in 3 ml of buffer (10 mM Tris, pH 8.0, 20 mM $MgCl_2$). Phage were purified from this suspension by centrifugation in cesium chloride block gradients, and the phage DNA was extracted by using essentially those methods described by Miller (22).

Restriction endonuclease digests. DNA was prepared and digested with enzymes *Eco*RI (Boehringer) and *Pst*I (New England Biolabs) by using the methods of Davey and Pittard (6).

RESULTS

Selection of Tn*10* insertions close to *tyrR*$^+$. The λ vector λNK55 was used to obtain transpositions of Tn*10* to the chromosome of strain W3110. Over 3,000 tetracycline-resistant clones were pooled and used to propagate a generalized transducing P1 lysate. By selecting for cotransduction of the gene of interest along with resistance to tetracycline, Tn*10* insertions

near that gene can be isolated (17).

Some *tyrR*$^+$ strains are resistant to 5×10^{-3} M β-2-thienyl-DL-alanine, an analog of phenylalanine, whereas an isogenic *tyrR* mutant derivative is sensitive to β-2-thienyl-DL-alanine at the same concentration (Camakaris and Pittard; manuscript in preparation).

The strain JP2144 is *tyrR* and sensitive to β-2-thienyl-DL-alanine. The P1 lysate obtained above was used to transduce this strain to resistance to both tetracycline and β-2-thienyl-DL-alanine. Twenty-three transductants were purified and shown to be *tyrR*$^+$ by streaking on medium containing fluorotyrosine. Phage P1 was propagated on each of these strains and used to transduce JP2152. Tetracycline-resistant transductants were selected on nutrient medium and screened for the coinheritance of *tyrR*$^+$ and *trkE*$^+$. The 23 strains fell into five distinct groups according to the frequency of cotransduction of *tyrR*$^+$ and *trkE*$^+$ along with resistance to tetracycline. The frequency of cotransduction of *tyrR*$^+$ with resistance to tetracycline ranged from 5 to 86%. From only one strain, JP2769, was *tyrR*$^+$ cotransduced with Tn*10* at a frequency of 86%. This strain was chosen for the next step in the experiment.

Integration of λNK55 near *tyrR*$^+$. λNK55 is unable to lysogenize a cell by integration at the *att*λ locus. After infection, recombination between the Tn*10* insertion in λNK55 and the homologous Tn*10* insertion in strain JP2769 can occur and will result in integration of the prophage near *tyrR*$^+$ (Fig. 1). Five stable λNK55 lysogens of JP2769 were obtained (see above). These were unable to produce phage on induction because this strain is unable to suppress the prophage *O*(Am) mutation, but were partially temperature sensitive. For these lysogens, when plated on nutrient agar, approximately 0.2% of cells survived at 42°C. All survivors at 42°C tested had lost the prophage. Strain JP2801 was one of these lysogens.

The pair of strains JP2803 and JP2804, constructed from JP2769 and JP2801, respectively, were used as donors in an interrupted mating experiment to confirm the site of prophage integration by zygotic induction (see above). The episome KLF29 mobilizes the chromosome in an anticlockwise direction. Transfer of Tn*10* from the nonlysogenic donor JP2803 began after about 80 min. No similar mobilization of Tn*10* from JP2804 was detected; however, the titer of infective centers in this mating mixture increased sharply at approximately the same time (data not shown). Similar results were obtained in identical experiments performed with corresponding derivatives of the other four lysogens.

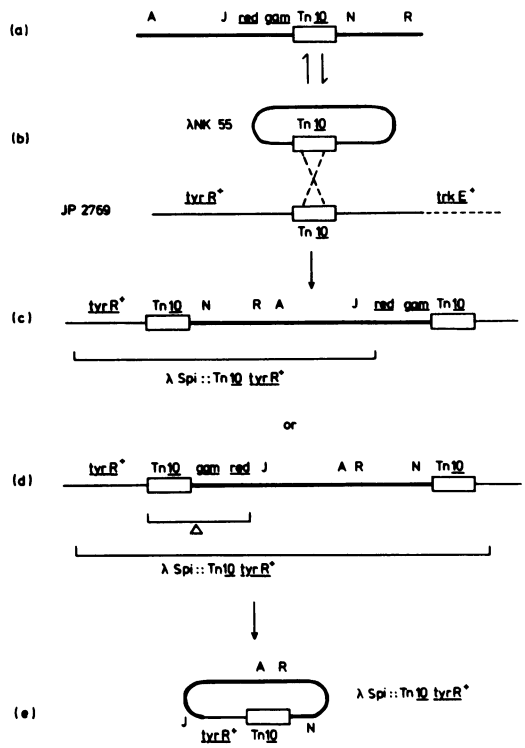

FIG. 1. *Isolation of λ(Tn10) tyrR*$^+$ *with λNK55. (a) The genome of λNK55 (not to scale). (b) λNK55 integration in the chromosome of JP2769 near tyrR*$^+$ *by homologous crossing-over between the two transposons to give (c) or (d), depending on the orientation of the chromosomal Tn10 insertion. An illegitimate recombination event on induction (c) or a Tn10-promoted deletion into the phage genome followed by an illegitimate recombination event on induction (d) can lead to the isolation of a specialized, λ Spi (Tn10) tyrR*$^+$ *transducing phage (e) (see text).*

Isolation of λ Spi (Tn*10*) *tyrR*$^+$. To enable strain JP2801 to produce phage on induction, the *supE* locus was introduced by phage P1 transduction from NK5012. On induction, a lysate of approximately 10^7 PFU/ml was obtained. By using this lysate and JP2144 as the recipient, transductants resistant to both β-2-thienyl-DL-alanine and tetracycline could not be obtained, (no transductants per 3×10^6 PFU), so the following scheme was used to enrich this lysate for possible plaque-forming λ *tyrR*$^+$ transducing phage.

The orientation of the Tn*10* insertion in JP2801 has not been determined. Integration of λNK55 into the chromosome via homologous recombination should, depending on the orientation of the Tn*10* inserted near *tyrR*$^+$, result in a prophage in one of two possible orientations with respect to the chromosome. These are

shown in Fig. 1c and d. Either of these orientations might give rise to λ(Tn*10*) *tyrR*$^+$ transducing phage which have been deleted for the *red* and *gam* loci. From one orientation (Fig. 1c), a one-step illegitimate recombination event could substitute the *red* and *gam* loci with chromosomal material, including *tyrR*$^+$. From the other (Fig. 1d), a two-step process would be required. Kleckner et al. (17) have reported that Tn*10* spontaneously excises from the chromosome, frequently causing deletions of adjacent chromosomal segments. A Tn*10*-promoted deletion of the *red* and *gam* loci with a subsequent illegitimate recombination event on induction could generate a transducing phage carrying both the remaining Tn*10* transposon and the *tyrR*$^+$ gene. These transducing phage may be defective.

Lindahl et al. (19) have observed that wild-type phage λ will not grow on *E. coli* strains lysogenic for phage P2 (Spi$^+$ phenotype). Many λ *bio*$^+$ transducing phage, for example, are able to grow on a P2 lysogen, and these are Spi$^-$. Expression of this phenotype requires defective δ, *red*, and *gam* genes (30).

Kleckner et al. reported λNK55 was able to plaque on a P2 lysogen (16) (strain NK5196). We found this phage unable to plaque on our P2 lysogen, JP2861 (Table 2). We have not investigated whether this apparent difference was due to the particular strains used or to some change in the λ phage; nevertheless, we predicted that deletion of the *red* and *gam* loci from λNK55 during the formation of a λ *tyrR*$^+$ transducing phage would render it Spi$^-$ with respect to JP2861.

A thermally induced lysate obtained from JP2802 was propagated on JP2861, thus enriching for any plaque-forming λ Spi. Transductants resistant to both β-2-thienyl-DL-alanine and tetracycline were obtained by using this lysate at a frequency of 5.6×10^{-6} per PFU. Of 28 transductant colonies, 13 yielded lysogens when streaked on nutrient plates seeded with a lawn of λ cI. When purified, these lysogens were found to be resistant to tetracycline and *tyrR*$^+$. The recipient strain, JP2144, is *sup*$^+$, and any derivatives of λNK55 should be *O*(Am). As expected, these lysogens were unable to produce phage on induction, and, as found previously, only about 0.2% of cells survived on plates at 42°C. Colonies surviving at 42°C were no longer lysogenic or tetracycline resistant, and some had become *tyrR*.

TABLE 2. *Efficiencies of plating of λ phage derivatives on phage P2 lysogenic and recA hosts*[a]

Phage	Efficiency of plating on:	
	P2 lysogen (JP2861)	*recA* (JP2862)
λNK55	$<1 \times 10^{-8}$	1.3
λ Spi (Tn*10*) *tyrR*$^+$	1.1	$<2 \times 10^{-4}$
λ Spi *chi* (Tn*10*) *tyrR*$^+$	0.71	$<3 \times 10^{-8}$

[a] All efficiencies of plating were measured at 37°C and are relative to plating on NK5012.

To allow phage production, the amber suppressor of NK5012 was introduced into one lysogen by P1 transduction. A thermally induced lysate from one *supE* derivative, JP2939, was used to transduce JP2144 with and without excess λ cI857. Transductants resistant to tetracycline were selected on nutrient medium at a frequency of 1.8×10^{-4} per PFU. All tetracycline-resistant lysogens selected from these transductant colonies had the *tyrR*$^+$ phenotype. The coinfection with excess λ cI857 increased the frequency of transduction 20-fold, and all tetracycline-resistant lysogens tested carried the λ cI857 prophage in addition.

We believe the low frequency of transduction reflects the low efficiency with which the infecting phage can stably integrate via homologous recombination. This hypothesis is supported by the finding that a helper phage increases the frequency of transduction.

From the lysate of JP2939, single plaques were purified and propagated. These were able to transduce JP2144 to resistance to tetracycline and *tyrR*$^+$ at frequencies similar to those previously obtained. For isolation of phage DNA, high-titer lysates are required; however, titers above about 10^8 PFU/ml could not be obtained with these phage.

Isolation of λ *chi* derivatives. Growing in a *recA* host, *red gam* phage mature an abnormally small fraction of the DNA they synthesize (10) and do not form plaques (30). On Rec$^+$ hosts, plaques are very small. If, however, a *chi* site is present in the phage DNA, large plaques are formed on a Rec$^+$ host (18, 21). A *chi* site may be present in the substituted chromosomal DNA of a transducing phage (20, 21), or a *chi* site may be acquired by mutation. Lam et al. (18) have pointed out that a *red gam* phage will yield large plaque-forming derivatives which have acquired a *chi* site by mutation after serial propagation through many generations.

One purified small plaque-forming λ Spi (Tn*10*) *tyrR*$^+$ phage from JP2939 was serially propagated on NK5012 until large plaque-forming derivatives were obtained. These were purified and propagated and were found to yield titers in excess of 10^{10} PFU/ml. These were also able to transduce resistance to tetracycline and *tyrR*$^+$ at frequencies similar to those previously obtained.

The efficiencies of plating of λNK55 and the λ Spi derivatives on *recA* and phage P2 lysogenic derivatives of NK5012 are shown in Table 2. λNK55 is able to plaque on a *recA* strain and not on a P2 lysogen, whereas the inability of the λ Spi derivatives to plaque on a *recA* strain confirms their Spi⁻ phenotype.

Restriction endonuclease analysis. Restriction endonuclease analysis of the DNA of λ wild-type, λNK55, and λ Spi *chi* (Tn*10*) *tyrR*+ was carried out by using the enzymes *Eco*RI and *Pst*I. The DNA fragments resolved by agarose gel electrophoresis are shown in Fig. 2.

The restriction fragment patterns of λNK55 can be predicted as follows: Tn*10* is 6.2 megadaltons (Md) in size and has a single asymetrically positioned *Eco*RI site and no *Pst*I sites (15). Insertion of Tn*10* at the site indicated by Kleckner et al. (16) will abolish *Eco*RI fragment B (4.7 Md) and *Pst*I fragment c (3.1 Md) (see Fig. 3 for lettering of fragments of λ wild-type) and create two new *Eco*RI fragments totalling 10.9 Md (i.e., 6.2 plus 4.7 Md). Depending on the orientation of the transposon, these two fragments should be about 5.5 Md each, or about 4 and 7 Md. Similarly, a new *Pst*I fragment of approximately 9.3 Md should be created by the insertion.

The *b*221 deletion should abolish *Eco*RI fragments A, D, and E and *Pst*I fragments b, d, h, and i. Given the coordinates of the deletion (7) and the standard fragment sizes used (27), theoretical values for the fusion fragments resulting from the deletion are 13.3 and 2.2 Md for *Eco*RI and *Pst*I, respectively. *Eco*RI fragments C and F and *Pst*I fragments a, e, f, g, j, and k should be retained along with smaller *Pst*I fragments not resolved on the gels.

Examination of the gels (Fig. 2) indicated that these fragments were retained. *Eco*RI fragment B and *Pst*I fragment c were absent. New *Eco*RI fragments were 7.2 and (assuming fragment D is abolished) 3.6 Md in size (totalling 10.8 Md), and a new *Pst*I fragment of 9.2 Md was also present. *Eco*RI fragments D and E appeared to be absent, and, assuming that fragment A was abolished, the new fusion fragment (13.7 Md) was approximately the same size as fragment A, compared with the predicted value of 13.3 Md. *Pst*I fragments b, d, i, and g or h were absent, as predicted, and a new fragment of 2.6 Md was present, compared with the predicted value of 2.2 Md. It appears, therefore, that the pattern of fragments obtained for both *Eco*RI and *Pst*I is consistent with the predictions made above. The relative positions of the *Pst*I and *Eco*RI cleavage sites of λNK55 are shown in Fig. 3b.

A comparison of the cleavage fragments of λNK55 and λ Spi *chi* (Tn*10*) *tyrR*+ DNA shows that in the formation of the latter, the 13.7- and

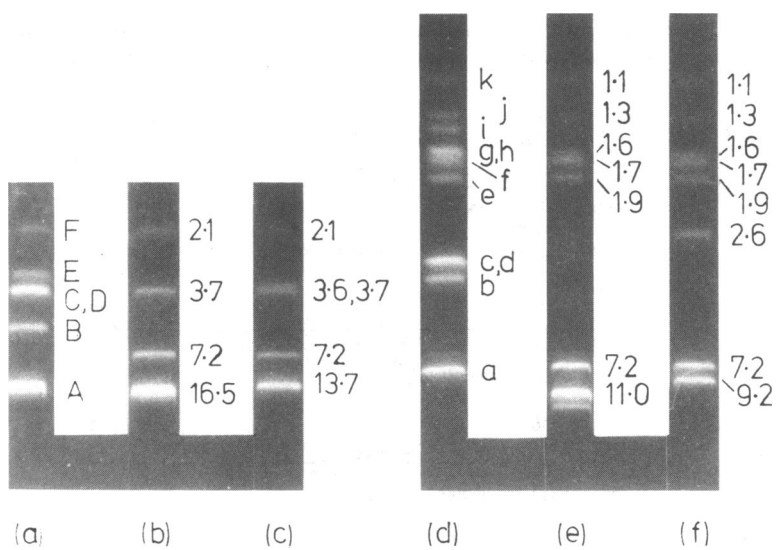

FIG. 2. *Restriction endonuclease cleavage fragments of λ DNAs. (a), (b), and (c) show EcoRI cleavage fragments of the DNAs of λ wild-type, λ Spi chi (Tn10) tyrR+, and λNK55, respectively, whereas (d), (e), and (f) show PstI cleavage fragments for the same DNAs, respectively. Standard fragment sizes used are those quoted by von Meyenberg et al. (27). Fragments of λ wild-type are lettered consecutively in order of decreasing size, with capital letters for EcoRI fragments and lower case for PstI fragments. Fragment sizes for λNK55 and λ Spi chi (Tn10) tyrR+ are shown in megadaltons.*

3.6-Md *Eco*RI fragments were abolished, resulting in a fusion fragment of about 16.5 Md, and the 2.6- and 9.2-Md *Pst*I fragments were abolished, resulting in a fusion fragment of 11.0 Md. These results indicate the absence in the λ *tyrR*$^+$ derivative of the *Eco*RI and the *Pst*I sites found immediately to the left of the Tn*10* insertion in λNK55. This confirms our prediction that the chromosomal DNA of the transducing phage would extend leftwards from the Tn*10* insertion, deleting the adjacent *red gam* region (Fig. 3c). It appears that the chromosomal DNA contains neither an *Eco*RI nor a *Pst*I cleavage site (nor a *Bam*HI site; data not shown).

The isolation of tetracycline-resistant nonlysogenic transductants by using λ(Tn*10*) *tyrR*$^+$ where, presumably, the Tn*10* has been transposed to a chromosomal location indicates that the transposon is intact, and although not all of the many *Pst*I fragments of the left arm (27) (Fig. 3) could be resolved on gels, this essential region is assumed to remain intact due to the plaque-forming nature of the phage.

DISCUSSION

We have described a general method for the generation of λ transducing phage in which homology between a chromosomal Tn*10* insertion and a phage λ(Tn*10*) is used to direct the integration of λ near the gene to be transduced. The method relies on a selection for the gene in question and the isolation of a Tn*10* insertion close enough to that gene to allow the formation of a transducing phage. The particular scheme used selects for the Spi$^-$ plaque-forming transducing phage. This imposes a limit on the size of the chromosomal substitution that can be accommodated in the phage genome.

Kleckner et al. (16) have indicated that the Tn*10* insertion of λNK55 is situated between the *c*III and *N* genes. DNA extending leftwards from the insertion up to gene *J* (i.e., the inessential *c*III, *kil*, *gam*, and *red* loci and the remainder of the *b* region) can be substituted to form a plaque-forming Spi$^-$ derivative of λNK55. From the map coordinates of the *J* and *c*III genes (8) and the coordinates of the *b*221 deletion (7), this inessential region of λNK55 amounts to about 9% of the wild-type λ genome. Up to 105% of the wild-type genome can be packaged efficiently (12), and the size of the λNK55 genome is only 98% of that of the wild type; therefore, an additional 7% can be accommodated along with the 9% that may be substituted. This amount of 16% can be equated with approximately 0.18 min of the *E. coli* chromosome (1, 7). According to Wu (29), this distance represents a cotransduction frequency of about 75%. Isolation of defective transducing phage would permit the use of a Tn*10* insertion more distant from the desired gene.

A recent report (16) suggests that Tn*10* is an unreliable marker in transductions by P1; however, this does not seem to be the case in this work. The maximum size of the chromosomal substitution in λ(Tn*10*) *tyrR*$^+$ (about 7% of the wild-type genome) is almost equivalent to the distance from the Tn*10* insertion to *tyrR*$^+$ in

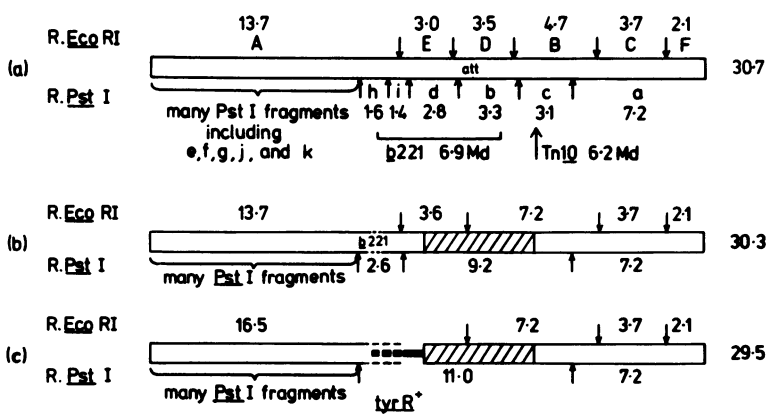

FIG. 3. *Restriction endonuclease cleavage maps of (a) λ wild type, (b) λNK55, and (c) λ Spi chi (Tn10) tyrR*$^+$. *λ DNA is represented by the open bars, and Tn10 is represented by the shaded bars. The left and right ends have been aligned for clarity. Cleavage sites for EcoRI and PstI are represented by vertical arrows above and below the bars, respectively. Fragment sizes are in megadaltons, and the sum of the EcoRI fragments is at the right end. Fragments of λ wild-type DNA are lettered consecutively in order of decreasing size (see Fig. 2). The size and extent of the b221 deletion and the point of insertion of the transposon Tn10 are indicated. att represents the phage attachment site, and the solid and dashed lines represent the minimum and maximum extents of the chromosomal (tyrR*$^+$*) segment, respectively.*

JP2760 predicted from the cotransduction frequency of 86%.

The gene $tyrR^+$ is known to produce a repressor protein which in combination with different effector molecules (i.e., the aromatic amino acids) controls the expression of a number of different loci on the chromosome involved in the biosynthesis of the aromatic amino acids (3, 9, 28). The λ $tyrR^+$ transducing phage described here will facilitate the cloning of the gene on a multicopy plasmid for subsequent physical analysis of both the gene and the gene product.

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