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Construction of a Single-Copy Promoter Vector and Its Use in Analysis of Regulation of the Transposon Tn*10* Tetracycline Resistance Determinant

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The construction and characterization of a promoter expression vector, λRS205, is described. λRS205 can be used for the in vitro construction of transcriptional (operon) fusions to the *lacZ* gene of *Escherichia coli* K-12. The level of β-galactosidase activity in lysogens of λRS205 fusion phages provides a quantitative measure of promoter function under single-copy conditions. The regulation of the Tn*10* tetracycline resistance gene (*tetA*) and the Tn*10* *tet* repressor gene (*tetR*) was examined by inserting DNA fragments that span the *tetR-tetA* promoter-operator region into λRS205. Levels of β-galactosidase in *tetA-lacZ* and *tetR-lacZ* fusion strains indicate that the *tetA* and *tetR* promoters are strong promoters; the *tetA* promoter is fourfold more active than the *tetR* promoter. Introduction of $tetR^+$ plasmids into *tetA-lacZ* and *tetR-lacZ* fusion strains represses β-galactosidase synthesis 15- to 60-fold and 6- to 15-fold, respectively. The concentration of tetracycline required to induce half-maximal β-galactosidase synthesis in these $tetR^+$ *tet-lac* strains depends on both the tetracycline resistance phenotype and the level of *tetR* repressor in the fusion strain. However, the induction of β-galactosidase in isogenic *tetA-lacZ* and *tetR-lacZ* strains is coordinate. The data presented here support the current model of Tn*10* *tet* gene organization and regulation and provide quantitative information about the regulation of *tetA* and *tetR* in vivo.

The construction of genetic fusions has provided a powerful tool for the analysis of transcription control signals in *Escherichia coli* and phage λ (4, 13, 14, 21, 39). Strategies for constructing genetic fusions differ as to: (i) the generation of transcriptional (operon) versus translational (gene) fusions; (ii) the choice of expressed function, e.g., *lacZ* or *galK*; (iii) in vivo versus in vitro construction; and (iv) the generation of multicopy (plasmid) versus single-copy (chromosomal) fusions. We describe the construction and characterization of a derivative of phage lambda, designated λRS205, that can be used for the in vitro construction of single-copy transcriptional fusions to *lacZ*.

The genetic organization of the Tn*10* tetracycline resistance determinant (*tet*) is shown in Fig. 1. Genetic and biochemical data indicate that the *tetA* resistance gene and the *tetR* repressor gene are transcribed from divergent promoters located between the *tetA* and *tetR* structural genes (6, 7, 29, 56). The *tetA* gene encodes a 43.2-kilodalton membrane protein that appears to be both necessary and sufficient for resistance to tetracycline (29, 34, 37, 49). The *tetR* gene encodes a 23.3-kilodalton protein that negatively regulates both its own synthesis and the synthesis of the TetA resistance protein (6, 28, 57, 58); tetracycline induces synthesis of both the TetA resistance protein and the TetR repressor (6, 34, 37, 56). The TetR repressor has been purified and shown to bind to appropriate *tet* DNA fragments in the absence, but not in the presence, of tetracycline (28). These obeservations have led to the proposal that the TetR repressor negatively regulates trascription of both the *tetA* and *tetR* structural genes (6, 56). We examined the regulation of *tetA* and *tetR* by constructing λRS205 derivatives that contain *tetA-lacZ* and *tetR-lacZ* transcriptional fusions.

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MATERIALS AND METHODS

Strains, phages, and plasmids. The *E. coli* K-12 strains, phages, and plasmids referred to below are described either in Table 1 in this section. B2550 and B2552 (Table 1) were constructed as follows: F′ *proAB* Δ*lacS20* was introduced into nalidixic acid-resistant derivatives of R^+EA2 and R^-EA2 (Table 1) by conjugation with E9001 (F′ *proAB* Δ*lacS20*/Δ[*lac-proAB*]XIII *supE*, from J. Beckwith), the mating mixture was spread on lactose tetrazolium agar containing nalidixic acid, and Lac^- homogenotes were isolated (44).

Media. LB broth contains 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 10 g of NaCl, and 5 g of yeast extract (Difco) per liter. T agar contains 10 g of tryptone, 8 g of NaCl, and 12 g of Bacto-Agar (Difco) per liter. TYE agar is T agar supplemented with 5 g of yeast extract per liter. CA agar is M63 minimal salts medium (44) with 0.4% glucose, 0.3% Casamino Acids (Difco), thiamine (2.5 μg/ml), 0.004 M sodium citrate, and Bacto-Agar (12 g/liter). Lactose tetrazolium agar and lactose MacConkey agar are described by Miller (44). XG (5-bromo-4-chloro-3-indolyl-β-D-galactoside; Bachem, Inc., Torrance, Calif.) was dissolved in *N,N*-dimethyformamide and added to T agar, TYE agar, and CA agar at a final concentration of 40 μg/ml. Colicin E1-resistant ($ColE1^r$) transformants were selected on TYE agar supplemented with 0.5% desoxycholate; colicin E1 was spread on the surface of the agar before use. TYE agar was supplemented with antibiotics as follows: ampicillin (100 μg/ml), nalidixic acid (50 μg/ml), neomycin sulfate (20 μg/ml), and tetracycline hydrochloride (20 μg/ml).

Enzymes. Restriction enzymes were purchased from New England Biolabs (Beverly, Mass.) and Bethesda Research Laboratories (Gaithersburg, Md.). T4 DNA ligase was purchased from New England Biolabs. Ligations were per-

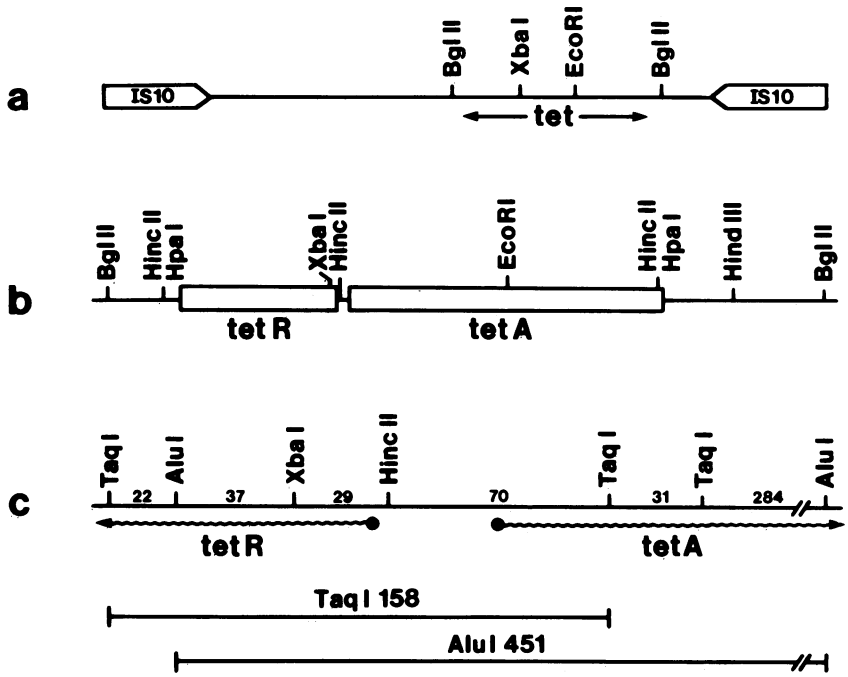

FIG. 1. Structure of the Tn*10 tet* region is 9,200 bp; the central region is flanked by 1,330-bp IS*10* sequences (open bars) in opposite orientations. (a) Tn*10*. (b) The 2,790-bp *Bgl*II fragment spans the structural genes for the TetR repressor and the TetA resistance proteins (open bars). (c) The 158-bp *Taq*I fragment and the 451-bp *Alu*I fragment span the *tetR-tetA* promoter-operator region. Wavy lines represent *tetR* and *tetA* RNA; the principal in vivo transcription initiation sites for *tetR* and *tetA* are separated by 36 bp (7). The sizes of restriction fragments are based on DNA sequence data (7, 29, 49).

formed in 10- to 25-μl volumes for 12 to 16 h at 15°C; the reactions contained 0.5 to 1.0 μg of DNA fragments and T4 DNA ligase in ligase buffer (20 mM Tris-hydrochloride, pH 7.6, 10 mM $MgCl_2$, 1 mM dithiothreotol, 1 mM Na_2 EDTA, and 50 μM ATP). *Micrococcus luteus* DNA polymerase I was a generous gift of R. D. Wells. DNA polymerase fill-in reactions were performed in 20-μl volumes for 1 h at 15°C; the reactions contained 0.5 to 1.0 μg of DNA fragments and *M. luteus* DNA polymerase in ligase buffer containing 10 μM each of dATP, dCTP, dGTP, and dTTP (25).

Construction of λRS205. The *Eco*RI and *Sal*I sites in λ and *lacZ* were manipulated to generate a phage with unique *Eco*RI and *Sal*I sites between the *trpA*$^+$-*lacZ*$^+$ region of λW205 (Table 1) and the λ *att* site. A mutation in the *Eco*RI site in the 3′ end of the *lacZ* gene (corresponding to β-galactosidase amino acids 1,006 and 1,007 [35]) was obtained in the phage Charon 16 (9) by alternating cycles of Charon 16 growth on RY13 (which carries the *Eco*RI restriction modification system; from F. Blattner) and X7026 (Δ[*lac-proAB*]XIII *supE*, from J. Beckwith). The resulting phage, λX616 (Charon 16 *sRIlacZ*°), like Charon 16, forms dark-blue plaques on X7026 on T-XG agar. The *sRIlacZ*° mutation was then introduced into λW205 as follows: (i) λX616J, an extended host range derivative of λX616, was selected by plating λX616 on a λ^r strain (CR63, from F. Blattner); (ii) X7026 was coinfected with λX616J and λW205; and (iii) *trpA*$^+$ recombinant phages carrying the λX616J host range mutation (presumably in the *J* gene) were selected on a λ^r *trpA* strain (CR63 Δ[*tonB-trpA*]553, this work) on 4-XG agar and then screened for the loss of the *Eco*RI site in *lacZ*. The recombinant phage is designated λW205JR (λW205 *J sRIlacZ*°).

Wild-type lambda (λ$^+$) has two *Sal*I sites (67.5% and 68.5% λ$^+$); the 499-base pair (bp) internal *Sal*I fragment spans *gam* and a portion of *bet* (18). A deletion that removes these two *Sal*I sites was obtained in the phage λW2001 (λ Δ[*sRIλ1-2*] *sRIλ3*° *cIam sRIλ4*° *sRIλ5*°, from F. Blattner) by annealing the cohesive ends of λW2001 DNA, digesting with *Sal*I, incubating with *M. luteus* DNA polymerase and deoxynucleotide triphosphates, religating with T4 DNA ligase, and transfecting K802 (*galK lacY met supE hsr*, from F. Blattner). The resulting phage, λW2001SP (λW2001 Δ[*bet-cIII*]), has no *Sal*I sites; it contains a 550- to 600-bp deletion that removes the 499-bp λ *Sal*I fragment and 50 to 100 bp of adjacent λ DNA. Since this deletion appears to reduce the efficiency of λRS205 lysogen formation (J. Little, personal communication), we presume it extends into *cIII*, the 3′ end of which is 58 bp from the *Sal*I site at 68.5% λ$^+$ (18).

*Eco*RI and *Sal*I sites were introduced 5′ to the *trpA*$^+$-*lacZ*$^+$ region of λW205JR (λW205 *J sRIlacZ*°) by constructing a plasmid intermediate. This plasmid, pRS205, is a derivative of the mini-ColE1 plasmid pVH51 (27); the 380-bp *Eco*RI-*Kpn*I fragment of pVH51 was replaced by two fragments, an 820-bp *Eco*RI-*Hin*dIII linker fragment from the plasmid pJG10 (38) and a 6,000-bp *Hin*dIII-*Kpn*I fragment from the phage λW205JR. The 820-bp *Eco*RI-*Hin*dIII fragment from pJG10 consists of a 135-bp *Eco*RI-*Hin*dII segment of pVH51 and a 685-bp *Hae*III-*Hin*dIII segment that spans the promoter-attenuator region of the threonine (*thr*) operon of *E. coli*; the *thr* promoter is oriented toward the *Eco*RI end of the fragment. This 820-bp fragment was selected because it contains a *Sal*I site 65 bp from its *Hin*dIII end. The 6,000-bp *Hin*dIII-*Kpn*I fragment from λW205JR spans the *trpA-lacZ* region of the phage; the *Hin*dIII site is in the 5′ end of *trpB* (corresponding to amino acids 30 through 32 of the TrpB protein [17]), the *lac*-λ junction is at 39.5% λ$^+$, and the *Kpn*I site is at 38.5% λ$^+$ (18). Therefore, the plasmid pRS205 is ColE1^r TrpA$^+$ LacZ$^+$ and has unique *Eco*RI and *Sal*I sites 5′ to the *trpA-lacZ* region.

TABLE 1. Strains, phages, and plasmids

Designation	Description or genotype	Source (reference)
Strain		
R⁺EA2	W3110 Δ*trpEA2 tna-2*	C. Yanofsky
R⁻EA2	W3110 Δ*trpEA2 tna-2 trpR*	C. Yanofsky
B2550	F′ *proAB ΔlacS20/ΔlacS20* Nal^r R⁺EA2	This work
B2552	F′ *proAB ΔlacS20/ΔlacS20* Nal^r R⁻EA2	This work
NK5031	Δ*lacM5265 supF* Nal^r	(43)
Phage		
λW205	λ *c*I857 *trp-lac* W205 Δ*trp*11/14-1c *S*am7	(3)
λRS205	LacZ⁺ *sRIlacZ*° *trpA*⁺ *sRIλ3*° Δ(*bet-c*III) *c*I857 *sRIλ4*° *nin5* *sRIλ5*°; single *Eco*RI and *Sal*I sites	This work
λplac5	*lacZ*⁺	(31)
Plasmid		
R100	Cm^r Sm^r Tc^r; Tn*10*	(33)
pRT29	ColE1^r Tc^r *tetR*⁺; 1,925-bp *Hpa*I Tn*10* fragment in pVH51	(34)
pRT210	ColE1^r Nm^r *tetR*⁺; 701-bp *Hin*cII Tn*10* fragment in pRZ112	(56)
pACYC177	Nm^r Ap^r	(15)
pBT402	Nm^r *tetR*⁺; 701-bp *Hin*cII Tn*10* fragment in pACYC177; *bla-tetR* transcription fusion	This work
pBT401	Nm^r *tetR*⁺; same as pBT402 except 701-bp *Hin*cII fragment in opposite orientation	This work

A recombinant phage, λRS205cIam, containing the *Eco*RI-*Sal*I-*trpA-lacZ* region of pRS205 and the rightward arm of λW2001SP (*sRIλ3*° Δ[*bet-c*III] *c*Iam *sRIλ4*° *sRIλ5*°) was constructed by (i) ligating a mixture containing an *Sst*I digest of λX616 (Charon 16 *sRIlacZ*°), an *Eco*RI digest of λW2001SP, and an *Sst*I-*Eco*RI digest of pRS205, (ii) transfecting YmelΔA (Δ[*tonB-trpA*]*553 supE* [4]), and (iii) selecting a TrpA⁺ LacZ⁺ recombinant phage on $\overline{\text{TRP}}$-XG agar. λX616 and pRS205 each have a single *Sst*I site in the middle of *lacZ*, corresponding to β-galactosidase amino acids 650 and 651 [35]); λW2001SP has a single *Eco*RI site (*sRIλ2*, 53.8% λ⁺) that is 1,620 bp to the left of the λ *att* site. The recombinant phage, λRS205cIam, consists of the leftward *Sst*I arm of the phage λX616 and the rightward *Eco*RI arm of the phage λW2001SP, joined by the *Sst*I-*Eco*RI fragment of the plasmid pRS205. Therefore, λRS205cIam has unique *Eco*RI and *Sal*I sites between the *trpA-lacZ* region and the λ *att* site. λRS205 (Table 1 and Fig. 2) is a *c*I857 *nin5* derivative of λRS205cIam; it was constructed by exchanging the rightward arms of λRS205cIam and λgt · λC (λ Δ[*sRIλ1-2*] *c*I857 *sRIλ4*° *nin5* *sRIλ5*° [53]). This was done by ligating *Xho*I digests of λRS205cIam and λgt · λC, transfecting K802, and selecting a LacZ⁺ recombinant phage carrying the *nin5* deletion of λgt · λC. There is one *Xho*I site (69.1% λ⁺) in both λRS205cIam and λgt · λC. Therefore, the recombinant phage, λRS205, has the *sRIλ2-att-sRIλ3*°-Δ(*bet-c*III)-*Xho*I region of λRS205cIam (and λW2001SP) and the *Xho*I-*c*I857-*sRIλ4*°-*nin5*-*sRIλ5*° region of λgt · λC. λRS205 is ca. 92% λ⁺ in length.

Construction of λRS205 derivatives. DNA fragments derived from the *lac* promoter-operator region, the *trp* promoter-operator-attenuator region, and the Tn*10* *tetR-tetA* promoter-operator region were inserted between the *Eco*RI and *Sal*I sites in λRS205. The cloning strategy was based on the observation that DNA polymerase "fill-in" of *Eco*RI (recognition sequence G ↓ AATTC) and *Sal*I (G ↓ TCGAC) fragments and subsequent ligation to blunt-ended fragments bearing 3′ G residues regenerates the *Eco*RI and *Sal*I sites (1, 55). Thus *Alu*I (AG ↓ CT), *Hae*III (GG ↓ CC), filled-in *Hpa*II (G ↓ CGC), and filled-in *Taq*I (T ↓ CGA) fragments were converted to *Eco*RI-*Sal*I fragments by ligation between filled-in *Eco*RI and *Sal*I sites. Promoter DNA fragments were inserted into λRS205 by one of two procedures: (i) The *tetA-lacZ* phage λRStet451-3 and the *tetR-lacZ* phage λRStet451-5 were constructed by a one-step procedure involving direct screening for promoter function (Lac⁺ plaque phenotype) in the recombinant phages. (ii) Other λRS205 derivatives were constructed by a two-step procedure involving the insertion of DNA fragments between the *Eco*RI and *Sal*I sites in pBR322 (11) and the subsequent transfer of the *Eco*RI-*Sal*I promoter fragments from these plasmid intermediates into λRS205. In general, it proved to be more efficient to verify the orientation of the DNA fragments and the regeneration of the *Eco*RI and *Sal*I sites in recombinant plasmids than in recombinant phages.

The pBR322-promoter plasmid intermediates were constructed by mixing 0.5 μg of pBR322 DNA (digested with *Eco*RI-*Sal*I) and an approximately fivefold molar excess of purified promoter DNA fragment, incubating with *M. luteus* DNA polymerase and deoxynucleoside triphosphates for 1 h, incubating with T4 DNA ligase for an additional 12 to 16 h, and transforming competent C600 SF8 (*thrB leu thi hsr hsm recB recC lop* [52]). Ampicillin-resistant (Ap^r) transformants were selected on TYE-ampicillin agar. In constructions involving *lac* promoter-operator fragments, Ap^r transformants were selected on TYE-ampicillin-XG agar, and blue colonies were picked and further analyzed (41). Plasmids in Ap^r transformants were initially characterized by size in agarose gels, and candidates were subsequently characterized by digestion with appropriate restriction enzymes. The properties of the plasmid intermediates are summarized below.

pRStet158-64 contains the 158-bp *Taq*I fragment that spans the Tn*10* *tetR-tetA* promoter-operator region (Fig. 1).

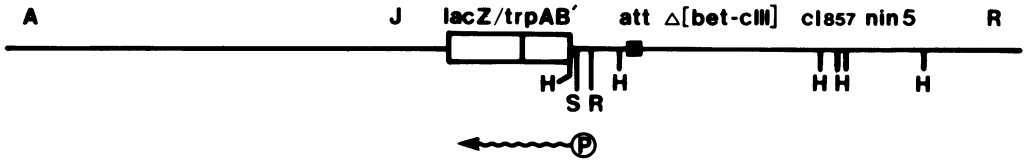

FIG. 2. Structure of λRS205. The W205 *trp-lac* substitution (open bar) extends from the λ *lac5* junction on the left to the *Hin*dIII site in *trpB* on the right. Δ(*bet-c*III) is a 550- to 600-bp deletion that removes the two *Sal*I sites in the *bet-gam* region of lambda. Mutations in the *Eco*RI sites in *lacZ*, *exo*, *O*, and near *S* are not shown. λRS205 has six *Hin*dIII sites (H), one *Sal*I site (S), and one *Eco*RI site (R). Insertion of appropriately oriented, promoter-containing DNA fragments into the *Sal*I-*Eco*RI region of λRS205 generates transcriptional fusions to *lacZ* (wavy line).

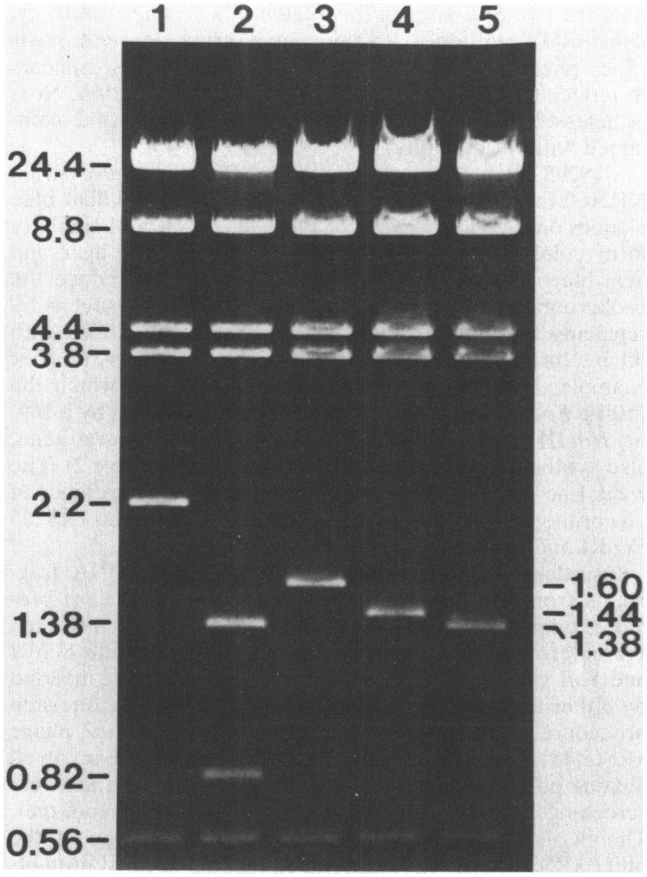

FIG. 3. Restriction digests of λRS205 and the *tetA-lacZ* phage λRStet158-43 electrophoresed in a 1% agarose gel. Lane 1, λRS205 with *Hin*dIII; lane 2, λRS205 with *Hin*dIII-*Eco*RI; lane 3, λRStet158-43 with *Hin*dIII; lane 4, λRStet158-43 with *Hin*dIII-*Eco*RI; lane 5, λRStet158-43 with *Hin*dIII-*Xba*I. Sizes of restriction fragments are given in kilobase pairs. The order of the *Hin*dIII restriction fragments in the physical map of λRS205 (Fig. 2) is: 24.4, 2.2, 8.8, 0.56, [0.13], 3.8, 4.4.

The *tet* fragment was purified from a *Taq*I digest of plasmid pRT29 (Table 1); the *Taq*I-*Sal*I junction in pRStet158-64 corresponds to amino acid 4 of the predicted TetA protein sequence (29, 49).

pRStrp570-15 contains the 570-bp *Hpa*II fragment that spans the promoter-operator-attenuator region of the *E. coli* *trpEDCBA* operon (36). The *trp* fragment was purified from a *Hpa*II-*Eco*RI digest of plasmid pVH153 (26), which consists of a 7,200-bp *Eco*RI *trpPOED* fragment inserted into pVH51 (27). The *Hpa*II-*Sal*I junction in pRStrp570-15 corresponds to amino acid 33 of anthranilate synthetase (*trpE*).

pRSlac95-16 and pRSlac203-5 contain the 95-bp *Alu*I fragment and the 203-bp *Hae*III fragment that span the promoter-operator region of the *E. coli lacZYA* operon (19, 35). The *lac* fragments were purified from *Alu*I and *Hae*III digests of plasmid pRZ3000 (41), which consists of a 789-bp *Hin*dII *lacP*$^+$ fragment inserted into pVH51. The *Alu*I-*Sal*I junction in pRSlac95-16 corresponds to nucleotide 36 of the *lac* transcript; β-galactosidase translation initiates at nucleotide 39. The *Hae*III-*Sal*I junction in pRSlac203-5 corresponds to amino acid 7 of β-galactosidase.

pRSlac95-216 and pRSlac203-6 are analogous to pRSlac95-16 and pRSlac203-5, except that the *lac* promoter fragments were purified from plasmid pRZ3UV5 (41), which is like pRZ3000 except that it carries the L8 mutation in the CAP-binding site (20) and the *UV5* mutations in the −10 region of the *lac* promoter (23). The *L8UV5 lac* promoter is CAP independent.

pRSlac169-3 contains a 169-bp *Hae*III fragment from within the 5′ end of the *lacZ* structural gene (35). The *lac* fragment was purified from a *Hae*III digest of plasmid pRZ3000; the *Hae*III-*Sal*I junction corresponds to amino acid 136 of β-galactosidase.

*Eco*RI-*Sal*I promoter fragments were shuttled from the pBR322-promoter plasmids into λRS205 by mixing 0.5 μg of λRS205 DNA and 0.5 μg of pRS plasmid DNA, digesting the mixture with *Eco*RI-*Sal*I, inactivating the *Eco*RI-*Sal*I (10 min, 70°C), ligating with T4 DNA ligase, and transfecting competent K802. The structures of progeny phages were screened by *Hin*dIII digestion of phage DNA prepared from plate lysates (12). Transfer of *Eco*RI-*Sal*I fragments from plasmids into λRS205 was very efficient; more than 50% of the phages recovered after ligation and transfection had the desired structure.

The λRS205 derivatives λRStet451-3 and λRStet451-5 were constructed by a one-step procedure as follows: The 451-bp *Alu*I fragment that spans the Tn*10* *tetR-tetA* promoter-operator region (Fig. 1) was purified from an *Alu*I digest of plasmid pRT29 (Table 1). The cohesive ends of 1 μg of λRS205 DNA were ligated with T4 DNA ligase before digestion with *Eco*RI-*Sal*I. The λRS205 DNA and 1 μg of purified 451-bp *Alu*I fragment (ca. 100-fold molar excess of *Alu*I fragment) were incubated with *M. luteus* DNA polymerase and deoxynucleoside triphosphates (1 h, 15°C) before addition of T4 DNA ligase (2 h, 20°C). The ligation mixture was packaged in vitro as described by Blattner et al. (8), except that the incubation of the ligated DNA with purified protein A and sonic extract was extended from 15 to 45 min. The packaged phage lysate was plated on the *lacZ* strain NK5031 on lactose MacConkey agar, and the structures of several Lac$^+$ phages (red plaques) were analyzed by digestion of phage DNA with *Hin*dIII, *Eco*RI, *Sal*I, and *Xba*I (Fig. 3). The *tetA-lacZ* phage λRStet451-3 contains a 455-bp *Eco*RI-*Sal*I fragment; the position of the *Xba*I site within the *Eco*RI-*Sal*I fragment indicates that the *tetA* promoter is oriented toward the *Sal*I site; the *Alu*I-*Sal*I junction corresponds to amino acid 99 of the predicted TetA protein sequence (29, 49). The *tetR-lacZ* phage λRStet451-5 also contains a 455-bp *Eco*RI-*Sal*I fragment; the position of the *Xba*I site indicates that the *tetR* promoter is oriented toward the *Sal*I site; the *Alu*I-*Sal*I junction corresponds to amino acid 15 of the predicted TetR protein sequence (7). The properties of these λRS205-promoter phages are summarized in Tables 2 and 3. λRS205 derivatives have also been utilized in the analysis of *trpR* autoregulation (10), growth-rate-dependent regulation of rRNA and protein (46), and R plasmid copy number control (51).

Phage growth. High-titer lysates (>5 × 10^{10} phages per ml) of λRS205 and λRS205 derivatives were prepared by infecting the host strain MO (from J. Beckwith). A modification of the method of Blattner et al. (9) was used for large-scale lysates. A mixture containing 5 × 10^8 phages, 1.5 ml of an overnight MO culture (grown in LB broth supplemented with 0.2% maltose), and 10 mM $MgSO_4$ was incubated for 15 min at 37°C without shaking and then diluted into 300 ml of K medium in a 2-liter flask and incubated at 37°C with vigorous shaking until lysis occurred (4 to 8 h). K medium is M9 minimal salts medium (44) with 0.4% glucose, 0.2% maltose, 1.0% Casamino Acids (Difco), 10 mM $MgSO_4$, 0.1 mM $CaCl_2$, and thiamine (2 μg/ml).

TABLE 2. β-Galactosidase activity in lysogens of λRSlac and λRStrp fusion phages[a]

Strain	Lysogenic phage	Promoter[b]	DNA fragment[b]	β-Galactosidase activity[c]
R⁺EA2				2,040
B2550				0
B2552 (*trpR*)				0
B2550	λplac5			2,110
B2550	λRS205			22
B2550	λRSlac169-72	None	169-bp *Hae*III	34
B2550	λRSlac95-51	*lacP*	95-bp *Alu*I	87
B2550	λRSlac203-11	*lacP*	203-bp *Hae*III	280
B2550	λRSlac95-62	*lacPUV5*	95-bp *Alu*I	220
B2550	λRSlac203-21	*lacPL8UV5*	203-bp *Hae*III	340
B2550	λRStrp570-91	*trpP*	570-bp *Hpa*II	68
B2552 (*trpR*)	λRStrp570-91	*trpP*	570-bp *Hpa*II	2,580

[a] Cultures were incubated at 32°C in M9 minimal salts medium supplemented with 0.2% glucose, L-tryptophan (40 μg/ml), thiamine (4 μg/ml), 1 mM $MgSO_4$, and 1 mM isopropyl-β-D-thiogalactopyranoside.

[b] Promoter and DNA fragment inserted into λRS205. *lacP* and *trpP* are the promoters for the *E. coli lacZYA* and *trpEDCBA* operons, respectively. *lacP UV5* contains the *UV5* mutations in the −10 region of *lacP* (23). *lacPL8UV5* contains the *L8* mutation in the *lacP* CAP-binding site (20) and the *UV5* mutations.

[c] Expressed in units defined by Miller (44).

β-Galactosidase assays. Lysogens of λRS205 and λRS205 derivatives were isolated by spotting phages on lawns of B2550 or B2552 and streaking from the zones of lysis onto TYE-XG agar. B2550 and B2552 form white colonies on TYE-XG; lysogens of λRS205 derivatives form blue colonies on TYE-XG. Ca. 50% of the lysogens isolated in this manner contain multiple prophages. Lysogens with a single prophage can be identified by the *ter* excision test (24); superinfection with the *int*⁻ *red*⁻ heteroimmune phage Charon 7 (9) yields lysates that have 10- to 100-fold higher titers of λRS205 in the case of multiple lysogens. In practice, relative levels of β-galactosidase provide a reliable means for identifying lysogens with a single copy of the prophage. β-Galactosidase was assayed as described by Miller (44). Lysogens were grown to saturation, subcultured at 1:50, and assayed after three to four cell doublings. The culture media for β-galactosidase assays are specified in the figure legends and table footnotes. All manipulations with lysogens were at 32°C.

RESULTS

Properties of λRS205. λRS205 (Table 1 and Fig. 2) is a derivative of the *trpA*⁺-*lacZ*⁺ fusion phage λW205. The *Eco*RI and *Sal*I sites in λ and *lacZ* were manipulated by a series of in vivo and in vitro procedures to generate a phage with unique *Eco*RI and *Sal*I sites between the λW205 *trpA-lacZ* region and the λ *att* site. Existing mutations in the λ *Eco*RI sites in *exo*, *O*, and near *S* were incorporated into λRS205. A mutation in the *Eco*RI site in the 3′ end of *lacZ* was selected on the basis of increased phage plating efficiency on an *Eco*RI-producing host; this mutation appears to have little or no effect on β-galactosidase activity. An 820-bp *Hin*dIII-*Sal*I-*Eco*RI linker fragment, derived from the promoter-attenuator region of the threonine (*thr*) operon, was introduced between the *Hin*dIII site in the λW205 *trpB* gene and the λ *Eco*RI site 1,620 bp to the left of the λ *att* site; the *thr* promoter is oriented toward λ *att*. A 550- to 600-bp deletion in the *bet-gam-c*III region was introduced to eliminate the two *Sal*I sites in this region of λ. As a result of the Δ(*bet-c*III) deletion, λRS205 cannot grow on *recA* hosts (Fec⁻ phenotype [59]). The Δ(*bet-c*III) deletion also appears to reduce the efficiency of λRS205 lysogen formation. Nevertheless, stable λRS205 lysogens can be isolated and maintained without difficulty.

λRS205 forms colorless plaques on the *lacZ* strain NK5031 (Table 1) on lactose MacConkey agar and light-blue plaques on T-XG agar. λRS205 lysogens of NK5031 similarly form colorless colonies on lactose MacConkey agar and light-blue colonies on T-XG agar. Attempts to reduce the background level of β-galactosidase in λRS205 lysogens by replacing the 750-bp *Eco*RI-*Sal*I fragment in λRS205 with other "promoterless" fragments were unsuccessful. For example, lysogens of λRSlac169-72, a phage in which the 750-bp *Eco*RI-*Sal*I fragment of λRS205 is replaced by a 169-bp *Hae*III fragment from within the *lacZ* structural gene, also synthesize about 30 U of β-galactosidase (Table 2). The weak Lac⁺ phenotype of λRS205 may reflect the presence of one or more promoters in the λ *b* region between the λRS205 *Eco*RI and *att* sites (50).

Insertion of specific promoters into λRS205. DNA fragments from the *lac* promoter-operator region, the *trp* promoter-operator-attenuator region, and the Tn*10 tetR-tetA* promoter-operator region were inserted between the *Eco*RI and *Sal*I sites in λRS205. Promoter fragments were inserted by either a one-step or a two-step procedure. The one-step procedure, used in the construction of the *tetA-lacZ* phage λRStet451-3 and the *tetR-lacZ* phage λRStet451-5, involved ligating purified promoter fragments into λRS205 and direct screening for promoter function (Lac⁺ plaque phenotype). The two-step procedure, used in the construction of the other λRS205 derivatives, involved ligating purified promoter fragments between the *Eco*RI and *Sal*I sites in pBR322 and the subsequent transfer of the *Eco*RI-*Sal*I promoter fragments from these plasmid intermediates into λRS205. Lysogens of λRS205 derivatives containing *lac*, *trp*, and *tet* promoter fragments synthesize significantly higher levels of β-galactosidase than lysogens of λRS205 itself (Tables 2 and 3). As discussed below, lysogens of the *lacP-lacZ* fusion

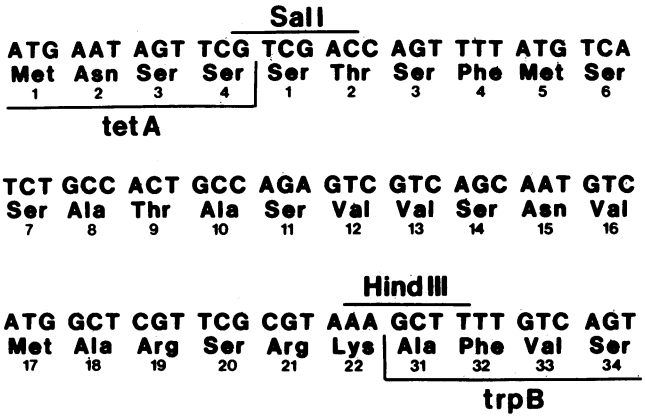

FIG. 4. Predicted nucleotide and amino acid sequences spanning the *tetA-trpB* junction region in λRStet158-43. Insertion of the 158-bp *Taq*I *tetA* promoter fragment into λRS205 generates a hybrid TetA-TrpB protein that consists of the amino-terminal four amino acids of TetA (7, 29, 49), a 22-amino acid segment encoded by the 65-bp *Sal*I-*Hin*dIII linker fragment (S. Lynn and J. Gardner, personal communication), and amino acids 31 through 397 of the TrpB protein (17).

phages synthesize lower levels of β-galactosidase than *lacZ*$^+$ control strains.

Efficiency of *lacZ* expression in λRS205 fusion phages. The properties of the λRS205 derivatives that we have constructed suggest that the level of *lacZ* expression in lysogens of these phages depends on the restoration of translation through the *trpB* region in λRS205, as well as the strength of the promoter inserted between the *Eco*RI and *Sal*I sites. λRS205 contains about 1,100 bp of the *trpB* structural gene, corresponding to amino acids 31 through 397 of the TrpB protein (Fig. 2). The promoter fragments inserted into the *trpE-lacZ* phage λRStrp570-91, the *tetA-lacZ* phage λRStet158-43, and the *tetR-lacZ* phage λRStet451-5 create in-frame translational fusions to the λRS205 *trpB* gene, and lysogens of these phages synthesize high levels of β-galactosidase (Tables 2 and 3). For example, the DNA sequence across the *tetA-Sal*I-*Hin*dIII-*trpB* junction in λRStet158-43 predicts the synthesis of a fusion protein that consists of amino acids 1 through 4 of the TetA protein, a 22-amino acid linker, and amino acids 31 through 397 of the TrpB protein (Fig. 4). In contrast, the predicted translation reading frames across the *tetA-Sal*I junction in λRStet451-3 and the *lacZ-Sal*I junctions in λRSlac203-11 and λRSlac203-21 are shifted +1, with the result that translation presumably terminates at the UAA codon in the region corresponding to TrpB amino acids 49 and 50 (17). The *lac* promoter fragments in λRSlac95-51 and λRSlac95-62 do not even include the *lacZ* translation initiation signal (19). The six- to eightfold difference in β-galactosidase activity between λplac5 and λRSlac203 lysogens (Table 2) and the sevenfold difference between λRStet158-43 and λRStet451-3 lysogens (Table 3) suggest that failure to translate the *trpB* region in λRS205 has a polar effect on *trpA-lacZ* expression.

Regulation of the *tetA* and *tetR* promoters. Lysogens of efficient *tetA-lacZ* and *tetR-lacZ* fusion phages (λRStet158-43 and λRStet451-5, respectively) synthesize high levels of β-galactosidase (Table 3), indicating that the *tetA* and *tetR* promoters are relatively strong. To examine the regulation of *tetA* and *tetR* by tetracycline and the TetR repressor, *tetR*$^+$ plasmids (Table 1) were introduced into the *tetA-lacZ* and *tetR-lacZ* lysogens. The *tetR*$^+$ plasmids R100, pRT29, pRT210, pBT401, and pBT402 differ in the degree to which they repress β-galactosidase levels in *tetA-lacZ* and *tetR-lacZ* strains (Table 3) and in the levels of tetracycline required to overcome this repression (Fig. 5).

Differences in the extent of TetR-mediated repression probably reflect differences in the levels of plasmid-encoded repressor. The low-copy-number plasmid R100, in which *tetR* is expressed from the autoregulated *tetR* promoter, represses β-galactosidase 15-fold in the *tetA-lacZ* strain and 6-fold in the *tetR-lacZ* strain. In contrast, the multicopy plasmid pBT402, in which *tetR* is expressed from the constitutive β-lactamase promoter, represses β-galactosidase 60-fold in the *tetA-lacZ* strain and 15-fold in the *tetR-lacZ* strain. Moreover, if the data are corrected for the significant basal level of β-galactosidase seen in λRS205 lysogens, the extent of pBT402-mediated repression increases to 95-fold at the *tetA* promoter and 25-fold at the *tetR* promoter. In every case, repression of the *tetA* promoter exceeds repression of the *tetR* promoter.

A striking feature of the data presented in Fig. 5 is the degree to which *tetR*$^+$ *tet-lac* strains differ in their response to tetracycline. For example, the concentrations of tetracycline required to obtain half-maximal induction of β-galactosidase in the *tetA-lacZ* (R100) and *tetA-lacZ* (pRT210) strains differ by four orders of magnitude. These differences appear to reflect differences in both the tetracycline resistance phenotypes and in the levels of plasmid-encoded repressor in *tetR*$^+$ *tet-lac* strains. The pRT210-, pBT401-, and pBT402-containing fusion strains are sensitive to tetracycline and are presumably capable of actively accumulating tetracycline from the medium (22, 32). In contrast, the R100- and pRT29-containing fusion strains are resistant to tetracycline and have a mechanism for reducing the intracellular accumulation of tetracycline (2, 40).

The multicopy plasmids pRT210, pBT401, and pBT402 all contain the 701-bp *Hin*cII *tetR*$^+$ fragment of Tn*10* (Fig. 1); however, the Tc^S fusion strains carrying these plasmids differ significantly in their response to tetracycline (Fig. 5). Since the 701-bp *Hin*cII fragment does not contain the *tetR* promoter-operator region (7, 56), the expression of *tetR* in these three plasmids depends on promoters in the plasmid vectors. For example, pBT401 and pBT402 differ only in the orientation of the 701-bp *Hin*cII *tetR*$^+$ fragment in the *Hin*cII site of the β-lactamase gene. Expression of *tetR* in pBT402 is under the control of the β-lactamase promoter, and minicell analyses indicate that pBT402 directs the synthesis of significantly higher levels of repressor than either pBT401 or pRT210 (unpublished observations). Whereas β-galactosidase synthesis in the *tetA-lacZ* (pRT210) strain is induced at very low tetracycline concentrations (0.2 ng/ml), the high levels of repressor in pBT402-containing fusion strains make it impossible to achieve sufficiently high concentrations of tetracycline to induce β-galactosidase in these Tc^S strains (Fig. 5).

In spite of the plasmid-specific differences between *tetR*$^+$ *tet-lac* strains, isogenic *tetR*$^+$ *tetA-lacZ* and *tetR*$^+$ *tetR-lacZ* strains are, in fact, equally responsive to tetracycline (Fig. 5). For example, half-maximal induction of β-galactosidase requires about 0.002 μg of tetracycline per ml with the *tetA-lacZ* (pBT401) and *tetR-lacZ* (pBT401) strains and about 2.0 μg of tetracycline per ml with the *tetA-lacZ* (R100) and *tetR-lacZ* (R100) strains.

TABLE 3. β-Galactosidase activity in lysogens of λRStet fusion phages[a]

Lysogenic phage	Promoter[b]	DNA fragment[b]	Plasmid	β-Galactosidase activity[c]
λRS205				35
λRStet158-43	$tetP_A$	158-bp *Taq*I	pACYC177	4,830
			R100	340
			pRT29	140
			pRT210	170
			pBT401	155
			pBT402	86
λRStet451-3	$tetP_A$	451-bp *Alu*I	pACYC177	640
			R100	110
			pBT401	94
			pBT402	62
λRStet451-5	$tetP_R$	451-bp *Alu*I	pACYC177	1,240
			R100	180
			pBT401	105
			pBT402	84

[a] Cultures of B2550 containing the indicated plasmids and lysogenic phages were incubated at 32°C in LB broth. pACYC177-, pRT210-, pBT401-, and pBT402-containing cultures were supplemented with neomycin sulfate (40 μg/ml).

[b] Promoter and DNA fragment inserted into λRS205. $tetP_A$ and $tetP_R$ are the promoters for Tn*10* *tetA* and *tetR*, respectively.

[c] Expressed in units defined by Miller (44).

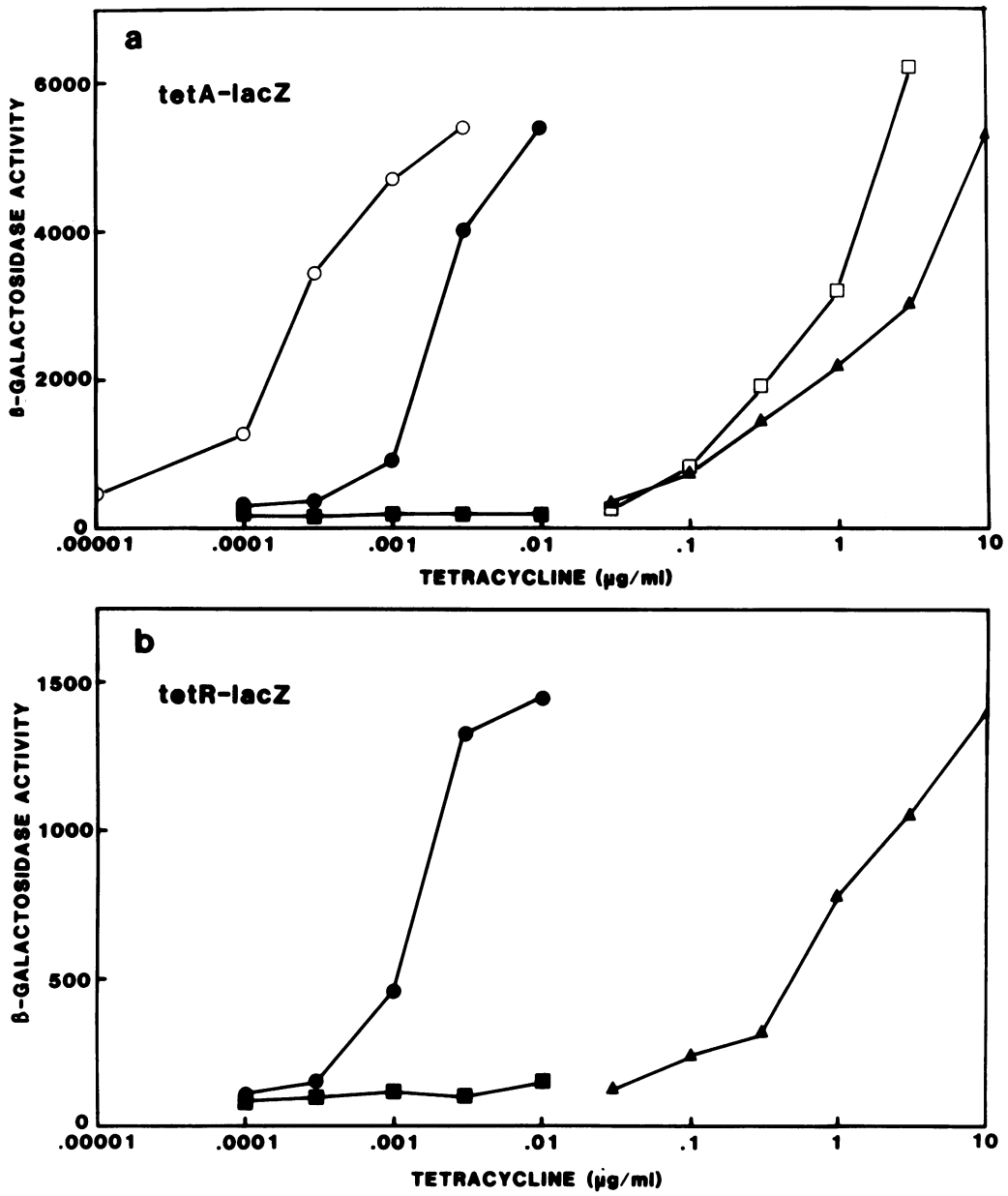

FIG. 5. Induction of β-galactosidase activity by tetracycline in lysogens of λRStet fusion phages. (a) Cultures of the *tetA-lacZ* fusion strain B2550 (λRStet158-43) containing the *tetR*$^+$ plasmids pRT210 (○), pBT401 (●), pBT402 (■), pRT29 (□), and R100 (▲) were incubated at 32°C in LB broth supplemented with the indicated concentrations of tetracycline. pRT210-, pBT401-, and pBT402-containing cultures were also supplemented with neomycin sulfate (40 μg/ml). (b) Cultures of the *tetR-lacZ* fusion strain B2550 (λRStet451-5) containing pBT401 (●), pBT402 (■), and R100 (▲) were treated as described in (a).

DISCUSSION

Other investigators have constructed transcriptional fusions to *lacZ* by taking advantage of the properties of the W205 *trp-lac* fusion (42, 43). The W205 fusion appears to be ideal for this purpose, since it fuses the 3′ untranslated region of the *trp* operon to the 5′ untranslated region of the *lac* operon, with the result that there is efficient transcription and translation of wild-type *lacZ* under the control of the *trp* promoter (3, 44a, 45). We constructed a derivative of phage λW205, designated λRS205, that is useful for the construction of transcriptional fusions to *lacZ*. Promoter-containing DNA fragments can be inserted between the unique *Eco*RI and *Sal*I sites in λRS205, and the β-galactosidase activity in lysogens of the resulting phages provides a measure of specific promoter function under defined single-copy conditions. Although plasmid vectors for constructing transcriptional fusions are, in general, easier to manipulate than phage vectors, plasmid copy number in these systems is extremely sensitive to growth conditions and the strength of the promoter being examined (C. Adams and G. W. Hatfield, personal communication).

The *Eco*RI-*Sal*I cloning strategy provides for the efficient and oriented transfer of *Eco*RI-*Sal*I promoter fragments from plasmids into λRS205. This design is specifically intended to facilitate the analysis of mutations produced in

plasmid subclones of regulatory sequences. DNA fragments generated by a number of different restriction enzymes can be converted to *Eco*RI-*Sal*I fragments by the action of DNA polymerase and DNA ligase, as described here. Alternatively, the polylinker sequences of plasmid vectors such as the pUC plasmids (54) can be exploited for this purpose.

The demonstration that β-galactosidase levels in *tetA-lacZ* and *tetR-lacZ* transcriptional fusion strains are repressed by $tetR^+$ plasmids and induced by tetracycline provides additional in vivo evidence that the expression of both *tetA* and *tetR* is regulated at the level of transcription by tetracycline and the TetR repressor (6, 56). These results are qualitatively similar to the results obtained previously with *tet-lac* translational fusion strains (5, 6). In addition, the high-level synthesis of β-galactosidase in lysogens of the *tetA-lacZ* phage λRStet158-43, and its repression by $tetR^+$ plasmids, confirms that the *tetA* promoter-operator region is within the 158-bp *Taq*I fragment that spans the region between the *tetA* and *tetR* structural genes (7, 28, 34, 56, 57). Lastly, the capacity of plasmid subclones carrying the 701-bp *Hin*cII fragment to regulate β-galactosidase synthesis in *tet-lac* strains confirms that the *tetR* structural gene is within the 701-bp *Hin*cII fragment (6, 16, 28, 56) and in addition provides a sensitive assay for the analysis of *tetR* and *tet* operator mutations (47, 57).

The levels of β-galactosidase in lysogens of efficient *tetA-lacZ* and *tetR-lacZ* transcriptional fusion phages provide a measure of the relative strengths of the *tetA* and *tetR* promoters. The data in Tables 2 and 3 suggest that the *tetA* promoter is more than twice as strong as the fully induced *lac* promoter under catabolite derepressing conditions. It is not surprising that the *tetA* promoter is a strong promoter; the DNA sequence of the *tetA* promoter is almost identical to the consensus sequence for *E. coli* promoters (7, 29). The level of β-galactosidase in lysogens of the *tetR-lacZ* phage λRStet451-5 indicates that the *tetR* promoter is also a relatively strong promoter. The *tetR* promoter is about one-fourth as strong as the *tetA* promoter and about one-half as strong as the *lac* promoter under catabolite derepressing conditions. Recent in vitro transcription studies suggest that *tetR* is transcribed from two overlapping promoters that are separated by about 20 bp (30). The relative contributions of these two promoters to *tetR* transcription in vivo is uncertain (see reference 7); however, if both promoters are active in vivo, β-galactosidase levels in λRStet451-5 lysogens should reflect the combined activities of both promoters.

The extent of TetR-mediated repression seen in *tet-lac* strains and the response of $tetR^+$ *tet-lac* strains to tetracycline are very much dependent on the source of repressor in the fusion strain (see also references 16 and 48). These differences appear to reflect two properties of the fusion strains: (i) the tetracycline resistance phenotype and (ii) the level of TetR repressor. In general, fusion strains that are sensitive to tetracycline, and are therefore capable of actively concentrating tetracycline from the medium (22, 32), can be induced to synthesize β-galactosidase with lower concentrations of the drug. The influence of the level of plasmid-encoded repressor is especially apparent among the $tetR^+$ *tet-lac* strains that are sensitive to tetracycline; lower levels of repressor significantly reduce the concentration of tetracycline required to induce β-galactosidase. However, isogenic $tetR^+$ *tetA-lacZ* and $tetR^+$ *tetR-lacZ* strains are equally responsive to tetracycline. This result is consistent with a model in which *tetA* and *tetR* transcription are coordinately regulated by repressor binding to shared operator sites that overlap the divergent *tetA* and *tetR* promoters (7, 29, 30, 57). The observations reported here provide further support for the current model of Tn*10 tet* gene organization and regulation and in addition provide quantitative data regarding the efficiencies of the *tetA* and *tetR* promoters in vivo and the regulation of the *tetA* and *tetR* promoters by tetracycline and TetR repressor.

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LITERATURE CITED

1. **Backman, K., M. Ptashne, and W. Gilbert.** 1976. Construction of plasmids carrying the *c*I gene of bacteriophage λ. Proc. Natl. Acad. Sci. U.S.A. **73**:4174–4178.
2. **Ball, P. R., S. W. Shales, and I. Chopra.** 1980. Plasmid-mediated tetracycline resistance in *Escherichia coli* involves increased efflux of the antibiotic. Biochem. Biophys. Res. Commun. **93**:74–81.
3. **Barnes, W. M., R. B. Siegel, and W. S. Reznikoff.** 1974. The construction of λ transducing phages containing deletions defining regulatory elements of the *lac* and *trp* operons in *E. coli*. Mol. Gen. Genet. **129**:201–215.
4. **Bassford, P., J. Beckwith, M. Berman, E. Brickman, M. Casadaban, L. Guarente, I. Saint-Girons, A. Sarthy, M. Schwartz, H. Shuman, and T. Silhavy.** 1978. Genetic fusions of the *lac* operon: a new approach to the study of biological processes, p. 245–261. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Beck, C. F.** 1979. A genetic approach to the analysis of transposons. Proc. Natl. Acad. Sci. U.S.A. **76**:2376–2380.
6. **Beck, C. F., R. Mutzel, J. Barbé, and W. Müller.** 1982. A multifunctional gene (*tetR*) controls Tn*10*-encoded tetracycline resistance. J. Bacteriol. **150**:633–642.
7. **Bertrand, K. P., K. Postle, L. V. Wray, Jr., and W. S. Reznikoff.** 1983. Overlapping divergent promoters control expression of Tn*10* tetracycline resistance. Gene **23**:149–156.
8. **Blattner, F. R., A. E. Blechl, K. Denniston-Thompson, H. E. Faber, J. E. Richards, J. L. Slightom, P. W. Tucker, and O. Smithies.** 1978. Cloning human fetal γ globin and mouse α-type globin DNA: preparation and screening of shotgun collections. Science **202**:1279–1284.
9. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L.-A. Furlong, D. G. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **196**:161–169.
10. **Bogosian, G., K. Bertrand, and R. Somerville.** 1981. Trp repressor protein controls its own structural gene. J. Mol. Biol. **149**:821–825.
11. **Bolivar, F., R. L. Rodriquez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. R. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
12. **Cameron, J. R., P. Philippsen, and R. Davis.** 1977. Analysis of chromosomal integration and deletions of yeast plasmids. Nucleic Acids Res. **4**:1429–1448.
13. **Casadaban, M., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:4530–4533.

14. **Casadaban, M., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–208.
15. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
16. **Coleman, D. C., and T. J. Foster.** 1981. Analysis of the reduction in expression of tetracycline resistance determined by transposon Tn*10* in the multicopy state. Mol. Gen. Genet. **182**:171–177.
17. **Crawford, I. P., B. P. Nichols, and C. Yanofsky.** 1980. Nucleotide sequence of the *trpB* gene in *Escherichia coli* and *Salmonella typhimurium*. J. Mol. Biol. **142**:489–502.
18. **Daniels, D. L., J. L. Schroeder, W. Szybalski, F. Sanger, and F. Blattner.** 1983. A molecular map of coliphage lambda, p. 467–517. *In* R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Dickson, R. C., J. N. Abelson, W. M. Barnes, and W. S. Reznikoff.** 1977. Genetic regulation: the *lac* control region. Science **182**:27–35.
20. **Dickson, R. C., J. Abelson, P. Johnson, W. S. Reznikoff, and W. M. Barnes.** 1977. Nucleotide sequence changes produced by mutations in the *lac* promoter of *Escherichia coli*. J. Mol. Biol. **111**:65–75.
21. **Franklin, N.** 1978. Genetic fusions for operon analysis. Annu. Rev. Genet. **12**:193–221.
22. **Franklin, T. J.** 1967. Resistance of *Escherichia coli* to tetracyclines. Changes in permeability to tetracyclines in *Escherichia coli* bearing transferable resistance factors. Biochem. J. **105**:371–378.
23. **Gilbert, W.** 1976. Starting and stopping sequences for the RNA polymerase, p. 193–205. *In* R. Losick and M. Chamberlain (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Gottesman, M. E., and M. B. Yarmolinsky.** 1968. The integration and excision of the bacteriophage lambda genome. Cold Spring Harbor Symp. Quant. Biol. **33**:735–747.
25. **Harwood, S., P. F. Schendel, and R. D. Wells.** 1970. *Micrococcus luteus* deoxyribonucleic acid polymerase. Studies of the enzymatic reaction and properties of the deoxyribonucleic acid product. J. Biol. Chem. **245**:5614–5624.
26. **Helinski, D. R., V. Hershfield, D. Figurski, and R. J. Meyer.** 1977. Construction and properties of plasmid cloning vehicles, p. 151–165. *In* R. F. Beers and E. G. Bassett (ed.), 10th Miles international symposium: impact of recombinant DNA molecules in science and society. Raven Press, New York.
27. **Hershfield, V., H. W. Boyer, B. L. Chow, and D. R. Helinski.** 1976. Characterization of a mini-ColE1 plasmid. J. Bacteriol. **126**:447–453.
28. **Hillen, W., G. Klock, I. Kaffenberger, L. V. Wray, Jr., and W. S. Reznikoff.** 1982. Purification of the TET repressor and TET operator from the transposon Tn*10* and characterization of their interaction. J. Biol. Chem. **257**:6605–6613.
29. **Hillen, W., and K. Schollmeir.** 1983. Nucleotide sequence of the Tn*10* encoded tetracycline resistance gene. Nucleic Acids Res. **11**:525–529.
30. **Hillen, W., K. Schollmeir, and C. Gatz.** 1984. Control of expression of the Tn*10*-encoded tetracycline resistance operon. II. Interaction of RNA polymerase and TET repressor with the *tet* operon regulatory region. J. Mol. Biol. **172**:185–201.
31. **Ippen, K., J. A. Shapiro, and J. R. Beckwith.** 1971. Transposition of the *lac* region to the *gal* region of the *Escherichia coli* chromosome: isolation of λ*lac* transducing bacteriophages. J. Bacteriol. **108**:5–9.
32. **Izaki, K., K. Kiuchi, and K. Arima.** 1966. Specificity and mechanism of tetracycline resistance in a multiple drug resistant strain of *Escherichia coli*. J. Bacteriol. **91**:628–633.
33. **Jacob, A. E., J. A. Shapiro, L. Yamamoto, D. I. Smith, S. N. Cohen, and D. Berg.** 1977. Plasmids studied in *Escherichia coli* and other enteric bacteria, p. 607–638. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Jorgensen, R. A., and W. S. Reznikoff.** 1979. Organization of structural and regulatory genes that mediate tetracycline resistance in transposon Tn*10*. J. Bacteriol. **138**:705–714.
35. **Kalnins, A., K. Otto, U. Ruther, and B. Muller-Hill.** 1983. Sequence of the *lacZ* gene of *Escherichia coli*. EMBO J. **2**:593–597.
36. **Lee, F., K. Bertrand, G. Bennett, and C. Yanofsky.** 1978. Comparison of the nucleotide sequence of the initial transcribed regions of the tryptophan operons of *Escherichia coli* and *Salmonella typhimurium*. J. Mol. Biol. **121**:193–217.
37. **Levy, S. B., and L. McMurry.** 1974. Detection of an inducible membrane protein associated with R-factor-mediated tetracycline resistance. Biochem. Biophys. Res. Commun. **56**:1060–1068.
38. **Lynn, S. P., J. F. Gardner, and W. S. Reznikoff.** 1982. Attenuation regulation in the *thr* operon of *Escherichia coli* K-12: molecular cloning and transcription of the controlling region. J. Bacteriol. **152**:363–371.
39. **McKenney, K., H. Shimatake, D. Court, U. Schmeissner, C. Brady, and M. Rosenberg.** 1982. A system to study promoter and terminator signals recognized by *E. coli* RNA polymerase, p. 383–415. *In* J. G. Chirkjian and T. Papas (ed.), Gene amplification and analysis: analysis of nucleic acids by enzymatic methods. Elsevier/North-Holland Biomedical Press, Amsterdam.
40. **McMurry, L., R. E. Petrucci, Jr., and S. B. Levy.** 1980. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:3974–3977.
41. **Maquat, L. E., and W. S. Reznikoff.** 1978. *In vitro* analysis of the *Escherichia coli* RNA polymerase interaction with wild-type and mutant *lac* promoters. J. Mol. Biol. **125**:467–490.
42. **Mauer, R., B. J. Meyer, and M. Ptashne.** 1980. Gene regulation at the right operator (O_R) of bacteriophage λ. I. O_R3 and autogenous negative control by repressor. J. Mol. Biol. **139**:147–161.
43. **Meyer, B. J., R. Mauer, and M. Ptashne.** 1980. Gene regulation at the right operator (O_R) of bacteriophage λ. II. O_R1, O_R2, and O_R3: their roles in mediating the effects of repressor and cro. J. Mol. Biol. **139**:163–194.
44. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

44a. **Ming, Y. X., L. Munson, and W. S. Reznihoff.** 1984. Molecular cloning and sequence analysis of *trp-lac* fusion deletions. J. Mol. Biol. **172**:355–362.

45. **Mitchell, D. H., W. S. Reznikoff, and J. R. Beckwith.** 1975. Genetic fusions defining *trp* and *lac* operon regulatory elements. J. Mol. Biol. **93**:331–350.
46. **Miura, A., J. H. Krueger, S. Itoh, H. A. de Boer, and M. Nomura.** 1981. Growth-rate-dependent regulation of ribosome synthesis in *E. coli*: expression of the *lacZ* and *galK* genes fused to ribosomal promoters. Cell **25**:773–782.
47. **Moyed, H. S., and K. P. Bertrand.** 1983. Mutations in multicopy Tn*10* *tet* plasmids that confer resistance to inhibitory effects of inducers of *tet* gene expression. J. Bacteriol. **155**:557–564.
48. **Moyed, H. S., T. T. Nguyen, and K. P. Bertrand.** 1983. Multicopy Tn*10* *tet* plasmids confer sensitivity to induction of *tet* gene expression. J. Bacteriol. **155**:549–556.
49. **Nguyen, T. T., K. Postle, and K. P. Bertrand.** 1983. Homology between the tetracycline resistance determinants of Tn*10* and pSC101. Gene **25**:83–92.
50. **Rosenvold, E. C., E. Calva, R. R. Burgess, and W. Szybalski.** 1980. *In vitro* transcription from the *b2* region of bacteriophage λ. Virology **107**:476–487.
51. **Rownd, R. H., A. M. Easton, and P. Sampathkumar.** 1981. Copy number control and incompatibility of *inc*FII R plasmids, p. 303–315. *In* S. B. Levy, R. C. Clowes, and E. L. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Press, New York.
52. **Struhl, K., J. P. Cameron, and R. D. Davis.** 1976. Functional expression of eucaryotic DNA in *Escherichia coli*. Proc. Natl.

Acad. Sci. U.S.A. **73**:1471–1475.
53. **Thomas, M., J. R. Cameron, and R. W. Davis.** 1974. Viable molecular hybrids of bacteriophage lambda and eucaryotic DNA. Proc. Natl. Acad. Sci. U.S.A. **71**:4579–4583.
54. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
55. **Wartell, R. M., and W. S. Reznikoff.** 1980. Cloning DNA restriction fragments with protruding single-stranded ends. Gene **9**:307–319.
56. **Wray, L. V., Jr., R. A. Jorgensen, and W. S. Reznikoff.** 1981. Identification of the tetracycline resistance promoter and repressor in transposon Tn*10*. J. Bacteriol. **147**:297–304.
57. **Wray, L. V., Jr., and W. S. Reznikoff.** 1983. Identification of repressor binding sites controlling expression of tetracycline resistance encoded by Tn*10*. J. Bacteriol. **156**:1188–1191.
58. **Yang, H. L., G. Zubay, and S. B. Levy.** 1976. Synthesis of an R plasmid protein associated with tetracycline resistance is negatively regulated. Proc. Natl. Acad. Sci. U.S.A. **73**:1509–1512.
59. **Zissler, J., E. R. Signer, and F. Schaefer.** 1971. The role of recombination in growth of bacteriophage lambda. I. The gamma gene, p. 455–468. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.