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Genetics

Construction of Tn*5 lac*, a transposon that fuses *lacZ* expression to exogenous promoters, and its introduction into *Myxococcus xanthus*

(gene expression/bacteriophage λ/bacteriophage P1/multicellular development/5-bromo-4-chloro-3-indolyl β-D-galactoside toxicity)

LEE KROOS AND DALE KAISER

Department of Biochemistry, Stanford University, Stanford, CA 94305

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ABSTRACT **A promoterless *trp–lac* fusion fragment was inserted near one end of the bacterial transposon Tn*5* in the correct orientation to fuse *lacZ* gene expression to promoters outside Tn*5*. The resulting transposon, Tn*5 lac*, retains the kanamycin-resistance gene of Tn*5* and transposes in *Escherichia coli* at 6% the frequency of Tn*5* to many different sites in a bacteriophage λ target. Expression of β-galactosidase, the product of the *lacZ* gene, from Tn*5 lac* insertions in phage λ depends both on insertion into a transcription unit in the correct orientation and on the regulation of the promoter of the transcription unit, verifying that by transposition Tn*5 lac* can fuse *lacZ* expression to outside promoters. An insertion of Tn*5 lac* in bacteriophage P1 was isolated and used to introduce Tn*5 lac* into *Myxococcus xanthus*, a bacterium that undergoes multicellular development. Stable kanamycin-resistant transductants are obtained that contain no P1 DNA sequences but have Tn*5 lac* inserted at different sites in the *Myxococcus* chromosome. Individual transductants express different levels of β-galactosidase. A chromogenic substrate of β-galactosidase, 5-bromo-4-chloro-3-indolyl β-D-galactoside, is toxic in *Myxococcus* when cleaved in large amounts. In principle, Tn*5 lac* could be used to assay transcription in any bacterium in which Tn*5* can transpose and β-galactosidase can be measured.**

Myxococcus xanthus is a Gram-negative soil bacterium that undergoes a primitive multicellular development with true cellular differentiation (1). During that developmental process, which depends on interactions between cells (2), many new gene products are synthesized (3). To identify genes that change their level of expression during development and to study the regulation of those genes, we wish to make operon fusions by transposition. A general approach, which uses transposition to fuse the expression of an easily assayed gene to promoters whose gene products are unknown or are not readily assayed, has been developed to identify and study interesting promoters (4).

Transposon Tn*5* can be introduced into *Myxococcus* from *Escherichia coli* by the specialized transducing phage P1::Tn*5* (5). It was previously found that bacteriophage P1 injects its DNA into *Myxococcus*, but the P1 is not stably maintained (6). Tn*5*, which encodes kanamycin resistance, can transpose from P1 to apparently random sites in the *Myxococcus* chromosome rendering cells stably kanamycin-resistant (Km^r) (5). Studies on the functional organization of Tn*5* had revealed that the left inverted repeat of Tn*5* (IS*50*L) is not needed to encode proteins required for transposition, but that some sequences near the left end of IS*50*L are required for transposition of Tn*5* (7). Therefore, it seemed feasible to insert a promoterless gene near the left end of Tn*5* to construct a transposon for making operon fusions. We chose to insert a promoterless *trp–lac* fusion fragment (8) into Tn*5* because it had been shown that the *lacZ* gene product, β-galactosidase, can be synthesized in *Myxococcus* and detected by cleavage of the standard chromogenic substrates (R. Gill and L. Avery, personal communication).

MATERIALS AND METHODS

Construction of Plasmids. Cloning operations and plasmid DNA purification were done as described (9). DNA polymerase I and T4 DNA ligase were gifts from S. Scherer. BAL-31 exonuclease (Bethesda Research Laboratories), calf alkaline phosphatase (Boehringer Mannheim), and phosphorylated linkers (Collaborative Research, Waltham, MA) were used according to the manufacturers' instructions. Plasmids were transformed (10) into *E. coli* MC1061 [*araD*139, Δ(*ara, leu*)7697, Δ*lacX*74, *galU*, *galK*, $hsdR^-$, $hsdM^+$, *strA*] (11).

Transposition Assay. Plasmids were transformed into BNN45 ($hsdR^-$, $hsdM^+$, *supE*44, *supF*, *thi*, *met*) (10), which contains the amber suppressor *supF* to permit propagation of λgt4 (Δ*lac5*, *c*I857, *nin5*, $S_{am}100$) (12) on LB plates (10) supplemented with 100 mM $MgSO_4$. The resulting phage stocks were titered for plaque formation on BNN45 at 37°C and for ability to form Km^r lysogens at 30°C when MC1061 was infected and plated on LB containing 30 μg of kanamycin sulfate per ml (Sigma) (10).

Analysis of Km^r λ Lysogens from the Transposition Assay. The amber mutation *S*100 in λgt4 facilitated analysis of Km^r lysogens obtained from the transposition assay by preventing lysis of cells by the *S* gene product. Lysogens were thermally induced to make phages and were lysed with chloroform (10). The lysates were titered for plaque formation on BNN45 growing on LB plates supplemented with 10 mM $MgSO_4$ at 37°C. Phage DNA was isolated from the lysogens by diluting (1:10) LB cultures grown at 30°C overnight into LB supplemented with 10 mM $MgSO_4$ and 30 μg of kanamycin per ml, growing for 3 hr at 37°C, and using a rapid plasmid isolation procedure (10). Transposon insertions in λgt4 were mapped by digesting phage DNA with appropriate restriction enzymes and by separating DNA fragments by horizontal gel electrophoresis on 0.5% agarose (MCB Chemical, Norwood, OH) gels (10).

To analyze β-galactosidase production by lysogens containing λgt4::Tn*5 lac*, LB cultures grown at 30°C overnight were diluted 1:25 into LB supplemented with 0.4% glucose/10 mM $MgSO_4$/and 30 μg of kanamycin per ml and grown for 2.5 hr at 30°C. Cells in the exponential growth phase were diluted into the same medium without kanamycin, to a density of 4×10^7 cells per ml, and β-galactosidase activity was quantitated in each of two cultures, one grown for 3 hr at 30°C and one shifted to 42°C for 0.5 hr followed by 2.5 hr at 37°C, using the *o*-nitrophenyl-β-D-galactoside (Calbiochem) cleavage assay (13).

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Abbreviations: Km^r, kanamycin-resistant; IS*50*L, the left inverted repeat of Tn*5*; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; kbp, kilobase pair(s); bp, base pair(s).

Isolation of P1::Tn*5 lac*. P1 (CM, *clr*100) (14) was used to infect MC1061 (pLRKΔ211) and a chloramphenicol-resistant lysogen was obtained at 30°C. A lysate, prepared by thermal induction of the lysogen (13), was used to infect SF800 [*polA*, *thy*, (Nal^r)] (15) (P1), and transductants resistant to chloramphenicol (25 μg/ml) (Sigma) and kanamycin (20 μg/ml) were selected at 30°C. These transductants were screened for sensitivity to colicin E1, which was prepared as described (16), by replica plating onto LB containing 1.4 μl of colicin E1 per ml, the minimum concentration lethal to colicin-sensitive cells. Colicin E1-sensitive transductants were thermally induced, the phages were purified on CsCl block gradients, and DNA was isolated by a formamide extraction procedure as described for λ (10).

Infection of *Myxococcus* with P1::Tn*5 lac*. SF800 (P1::Tn*5 lac*) was thermally induced (13) and phages were sedimented from the lysate by centrifugation at 6000 × *g* for 15 hr. The phage pellet was resuspended in 1/25th the original volume of buffer (10 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$). In a typical transduction, 200 μl of this concentrated phage stock was mixed with 1 ml of *M. xanthus* DK101 (*sgl*A1) (17) growing exponentially in CTT medium (17) to define CTT medium at 32°C at a density of 3–5 × 10^8 cells per ml; the mixture was made 5 mM $CaCl_2$ by addition from a 0.5 M stock, preincubated for 30 min at room temperature, then plated in 2.5 ml of CTT top agar on CTT plates containing 20 μg of kanamycin per ml. Plates were incubated at 32°C for 18–24 hr and then overlayed with 2.5 ml of CTT top agar containing enough kanamycin to make the final concentration in the plate 40 μg/ml. Colonies that grew after an additional 4–6 days at 32°C were transferred to CTT plates containing 40 μg/ml kanamycin and CTT plates containing 40 μg of 5-bromo-4-chloro-3-indolyl β-D-galactoside per ml (X-Gal) (Boehringer Mannheim).

RESULTS

Identification of Tn*5 lac*. The construction of Tn*5 lac* is outlined in Fig. 1. It was necessary to remove the transposase promoter, P_T, at 60–90 base pairs (bp) from the left end of Tn*5* (20), yet retain sequences required for transposition. The amount of sequence at the left end of Tn*5* required for transposition was unknown when we began these experiments, so BAL-31 exonuclease was used to generate a set of plasmids with deletions beginning at the *Bam*HI site 187 bp from the left end of Tn*5* and extending (counterclockwise) toward the *Eco*RI site in the ColE1 vector. To indicate when the transposase promoter had been deleted, two plasmids were constructed as standards. One, pLRK32, retains all 187 bp at the left end of Tn*5* and serves as the standard for an intact transposase promoter. The other, pLRK1, has the entire transposase promoter deleted by removal of the smaller *Eco*RI/*Bam*HI fragment of pLRK32. In the *E. coli lac*$^-$ strain MC1061, pLRK32 was found to display a higher level of β-galactosidase expression than pLRK1, as expected, because both transposase promoter and readthrough from vector promoter(s), P_V, (21) transcribe the *trp–lac* fusion segment in pLRK32, but only readthrough from vector promoter(s) remains in pLRK1. In strain MC1061, as shown in Table 1, deletion plasmids constructed by BAL-31 digestion (Fig. 1) displayed a Lac phenotype corresponding to one of the standards, either pLRK32 or pLRK1. The data reported in Table 1 are consistent with a transposase promoter located 60–90 bp from the left end of Tn*5*, because the Lac phenotype switches from that of pLRK32 for deletion 131, in which the plasmid retains about 150 bp of the left end of Tn*5*, to that of pLRK1 for deletion 211, in which the plasmid retains only about 30 bp and is thus predicted to have deleted the transposase promoter.

To determine whether transposability was retained for each deletion plasmid, phage λ was used as a target. Transposition from plasmid to λ was detected by the ability of transposon-carrying phage to transduce kanamycin resistance to *E. coli*. Two deletions in λ strain gt4 enable it to accept more than 12 kilobase pairs (kbp) of DNA (the expected length of Tn*5 lac*) and still be packaged in a phage head. Measurements of the transposition frequencies are shown in Table 1. Because deletion 211 had removed the transposase promoter at the left end of Tn*5* judging from the Lac phenotype, and yet gave rise to Km^r transductants at 6% the frequency of pRZ104, which contains Tn*5*, it was identified as the desired deletion and its transposon was designated Tn*5 lac*. It is unlikely that the lower transposition frequency of Tn*5 lac* relative to Tn*5* is due to deletion of sequences in IS*50*L important for transposition, because the transposons in pLRK 32 and pLRK21, which retain 187 bp at the left end of Tn*5* and IS*50*L intact, respectively, also transpose at 6% the frequency of Tn*5*. The lower transposition frequency of Tn*5 lac* may be due to the increased distance

FIG. 1. Structures of plasmids. Deletion plasmids were constructed by ligating fragment I from pLRK5412 to a set of fragments II generated from pLRK26 by digesting with *Bam*HI, treating with BAL-31 exonuclease for different amounts of time, filling in ends with DNA polymerase I, ligating phosphorylated *Bam*HI linkers on the blunt ends, and digesting with *Bam*HI and *Sal* I. The 680-bp *Eco*RI/*Bam*HI segment of fragments II is expanded to show the position of BAL-31-generated deletions (⇶) and the position and direction of transcription of the transposase promoter, (P_T)►. Other symbols are (P_V)►, vector promoter(s); Km, the kanamycin resistance gene of Tn*5*; ——, ColE1; ■, IS*50*L; ∿∿, the central region of Tn*5*; ▨, the right inverted repeat of Tn*5*; ▭, the *trp–lac* fusion fragment; R, *Eco*RI; B, *Bam*HI; S, *Sal* I; X, *Bam*HI site that was deleted. pLRK5412 had been constructed by subcloning the 3.1-kbp *Eco*RI/*Sal* I fragment of pRZ104 (18) into *Eco*RI/*Sal* I-digested pBR322 (19) to form pLRK718, filling in the *Hin*dIII site in IS*50*L of pLRK718 and converting it to a *Bam*HI site to form pLRK118, and inserting the 7.6-kbp *Bam*HI/*Bgl* II *trp–lac* fusion fragment of pMC903 (8) into *Bam*HI-digested pLRK118. pLRK26 had been constructed by digesting pRZ104 with *Bam*HI, filling in the ends, and ligating the blunt ends to form pLRK3, converting the *Hpa* I site in IS*50*L of pLRK718 to a *Bam*HI site to form pLRK731, and inserting the 3.1-kbp *Eco*RI/*Sal* I fragment of pLRK731 into *Eco*RI/*Sal* I-digested pLRK3. pLRK32 was constructed by ligating a mixture of *Bam*HI/*Sal* I-digested pLRK26 and pLRK5412 and identifying the recombinant in which fragment I was joined to fragment II without deletion. pLRK1 was constructed by deleting the 680-bp *Eco*RI/*Bam*HI fragment of pLRK26, leaving *Eco*RI and *Bam*HI sites, to form pLRK14, and ligating a mixture of *Bam*HI/*Sal* I-digested pLRK14 and pLRK5412. pLRK21 was constructed by deleting the 0.5 kbp *Sma* I/*Bam*HI fragment from pRZ104, leaving a *Bam*HI site in its place to form pLRK6, then inserting the 7.6-kbp *Bam*HI/*Bgl* II fragment from pMC903 into *Bam*HI-digested pLRK6 in the orientation in which *lacZ* expression becomes fused to the promoter of the kanamycin-resistance gene of Tn*5*.

Table 1. Identification of Tn*5* *lac*

Plasmid	Sequence remaining at left end, bp*	Relevant structure†	Lac phenotype‡	Transposition	
				Frequency§	% pRZ104
pRZ104	1534		–	5×10^{-6}	100
pLRK32	187	trp lac	++	3×10^{-7}	6
pLRKΔ131	150 ± 15	trp lac	++	3×10^{-7}	6
pLRKΔ211	30 ± 10	trp lac	+	3×10^{-7}	6
pLRKΔ514	0	trp lac	+	$<1 \times 10^{-9}$	<0.02
pLRK1	0	trp lac	+	$<1 \times 10^{-9}$	<0.02
pLRK21	1534	trp lac	+++	3×10^{-7}	6

*The amount of IS*50*L to the left of the *trp–lac* fusion fragment was determined for deletion plasmids from the size of the smaller *Eco*RI/*Bam*HI fragment as measured by gel electrophoresis with fragments of known sizes.

†The structure of the plasmid between the *Eco*RI site in ColE1 and the right end of the right inverted repeat of Tn*5* is shown. Symbols are the same as in Fig. 1 and construction of plasmids is described in the legend of Fig. 1.

‡+, ++, and +++, increasing levels of β-galactosidase expression in *E. coli* MC1061 on MacConkey lactose indicator plates (13).

§The transposition frequency is the number of Km^r lysogens obtained at 30°C per plaque-forming unit.

between its ends. Decreased transposition with increased distance between the ends of transposable elements has been observed for transposons bordered by IS*1* (22) or IS*10* (23).

No transposition was observed for deletion 514 or pLRK1, as expected, because the left end of Tn*5* was deleted from these plasmids. A careful deletion analysis of the left end of Tn*5* has shown that transposition is abolished when less than 16–18 bp of this end remain (24). Treating the λ phage stocks with deoxyribonuclease I to digest all DNA not protected by phage heads did not change the transposition frequency, while incubation of phage stocks with λ antiserum abolished both the ability to obtain Km^r transductants and the ability to form plaques. These results and restriction mapping of phage DNA from Km^r lysogens obtained from the transposition assay, which is described below, indicate that the frequency of Km^r transductants is a valid measure of the frequency of transposition from plasmid to λ.

Tn*5* *lac* Transposes to Many Sites in λ. Phage DNA was isolated from 11 Km^r lysogens obtained in the transposition assay of Tn*5* *lac*, and the position and orientation of each insertion was mapped by restriction analysis. Also, the position of 10 Tn*5* insertions in λgt4, obtained similarly, were mapped as controls and the results are shown in Fig. 2. The sites of Tn*5* *lac* insertions in λgt4 appear to be distributed as widely as those of Tn*5*. When the Km^r lysogens were thermally induced, all but one produced infective phage, although all insertions in the rightward transcripts reduced plaque size. The one Km^r lysogen that failed to produce plaque-forming phage appeared to have Tn*5* inserted in the *J* gene of λgt4. The manner in which the Km^r lysogens were obtained selected against insertions in regions of λgt4 that are essential for either lytic growth or lysogeny, which accounts for the low frequency of insertions that map in essential genes. All λgt4::Tn*5* *lac* formed tiny plaques on BNN45 growing on LB plates not supplemented with 10 mM $MgSO_4$, presumably because λgt4::Tn*5* *lac* phage heads are unstable in the absence of added Mg^{2+}. For this reason, plates were supplemented with 100 mM $MgSO_4$ for growth of λgt4 on plasmid-containing strains in the transposition tests reported in Table 1.

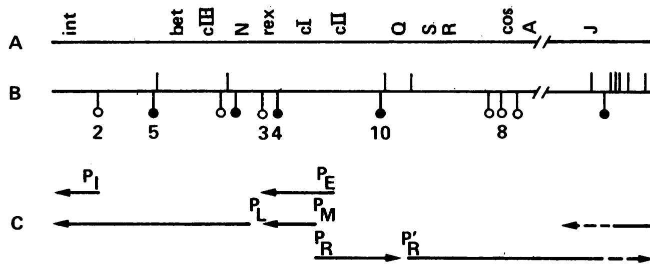

FIG. 2. Position of Tn*5* and Tn*5* *lac* insertions in λgt4 (*B*) in relation to its known gene (*A*) and transcription (*C*) maps. (*A*) Partial gene map of λgt4 prophage excluding most head and tail genes (–//–). (*B*) Tn*5* insertions (⊥) were mapped by digestion of phage DNA with *Bam*HI, *Sma* I, and *Bam*HI followed by *Eco*RI. Tn*5* *lac* insertions were mapped by digestion with *Bam*HI and *Eco*RI, allowing the orientation of Tn*5* *lac* to be determined as that in which the *trp–lac* segment could be expressed by rightward (⫱) or leftward (⫪) transcription. β-galactosidase activity was determined for the numbered insertions (see Table 2). (*C*) Positions of known transcription units in λgt4. ⟶, Extent and direction of transcription; --->, readthrough (25).

β-Galactosidase Expression from Tn*5* *lac* in λ Correlates with the Phage Transcription Map. Lysogens with different insertions of Tn*5* *lac* in λgt4 allowed testing of the ability of Tn*5* *lac* to fuse β-galactosidase expression to exogenous promoters, because the regulation of transcription units in λ is well known both for the lysogenic state and after induction of lytic development (25). For example, Tn*5* *lac* insertion 4 is in the *rex* gene in the correct orientation to fuse β-galactosidase expression to the P_M or P_E promoters (Fig. 2). Data in Table 2 show that β-galactosidase, like repressor and *rex*, is expressed in the strain 4 lysogen and that its expression falls when phage production is induced by transiently shifting the temperature of the (*c*I857) lysogen to 42°C. Insertion 3 is also

Table 2. β-Galactosidase expression from Tn*5* *lac* insertions in λ

Strain*	β-Galactosidase activity, units		Ratio§
	Lysogens†	After induction of λ‡	
wt	1100	1,100	1.0
4	1200	590	0.5
3	150	430	2.9
5	190	7,800	41
8	290	40,000	140
2	340	970	2.9
10	150	1,100	7.3

*The wild-type (wt) strain was MC1020 [*ara*D139, Δ(*ara*, *leu*)7697, *lac*$^+$, *galU*, *galK*, *strA*] (11); it was induced for β-galactosidase expression by including 1 mM isopropyl-thiogalactoside (Sigma) in the medium. Other strains were MC1061 (λgt4::Tn*5* *lac*) with Tn*5* *lac* inserted in the position and orientation indicated in Fig. 2.

†The strain was grown at 30°C.

‡The strain was grown at 30°C then shifted to 42°C for 0.5 hr followed by 37°C for 2.5 hr.

§The ratio of β-galactosidase activity in the culture after induction of λ to that in the lysogen.

in the *rex* gene, but in the wrong orientation to fuse β-galactosidase expression to P_M or P_E. It shows 1/8th as much expression in the lysogen as insertion 4. Insertions 5 and 8, which are in the correct orientation to fuse β-galactosidase expression to P_L and P'_R, respectively, show lower expression in their lysogens than insertion 4, with 41- and 140-fold increases in expression when the prophage is induced and phage growth ensues. Insertions 2 and 10, which are in the leftward and rightward transcription units (but in the wrong orientation to fuse β-galactosidase expression to P_L and P_R, respectively), display low levels of expression in their lysogens. The increase in β-galactosidase activity observed for insertions 3, 2, and 10 after induction of the prophage may be due to readthrough of antiterminated phage transcripts and the increased number of phage genomes. In summary, the level of expression of β-galactosidase from Tn*5 lac* insertions in λgt4 depends on the position and orientation of the Tn*5 lac* insertion and correlates with the known regulation of transcription units in λ.

Isolation of P1::Tn*5 lac*. To introduce Tn*5 lac* into *Myxococcus*, an insertion of Tn*5 lac* in phage P1 was first obtained. Transposition of Tn*5 lac* from pLRKΔ211 to P1 was detected by transduction into SF800 (P1). A P1 lysogenic recipient is necessary because insertion of Tn*5 lac* into P1 is expected to eliminate terminal redundancy and so prevent circularization of P1::Tn*5 lac* when it enters *E. coli* (26); thus, homologous recombination between linear P1::Tn*5 lac* and a (circular) resident P1 prophage can rescue the transposition product. The *polA* allele in SF800 was used to prevent maintenance of pLRKΔ211 that might be introduced by P1 generalized transduction. Another undesired event, P1–pLRKΔ211 cointegrate formation mediated by IS*1* or Tn*9* in P1, could be distinguished from Tn*5 lac* transpositions by screening transductants for colicin immunity encoded in the ColE1 portion of pLRKΔ211. Cointegrations gave rise to Km^r transductants at about the same frequency as transposition of Tn*5 lac* from pLRKΔ211 to P1.

Analysis of phage DNA from three colicin-sensitive transductants confirmed the insertion of Tn*5 lac* at different positions in P1. Structural analysis of only the P1::Tn*5 lac* used in further studies is shown in Fig. 3. Since Tn*5 lac* has a single *Eco*RI site 4 kbp from one end and 8 kbp from the other end, insertion of Tn*5 lac* into P1 should result in the disappearance of one normal P1 *Eco*RI restriction fragment and the appearance of two new fragments, one >4 kbp and one >8 kbp. The sum of the two new fragments should be 12 kbp longer than the missing fragment. The ethidium bromide-stained gel in Fig. 3*A* shows the expected pattern. In P1::Tn*5 lac*, a 6.5-kbp *Eco*RI restriction fragment is absent and two new fragments are present at 14 and at 4.5 kbp. Southern blot hybridization using pLRKΔ211 as the labeled probe (Fig. 3*B*) shows that only the new fragments contain Tn*5 lac* homology as expected.

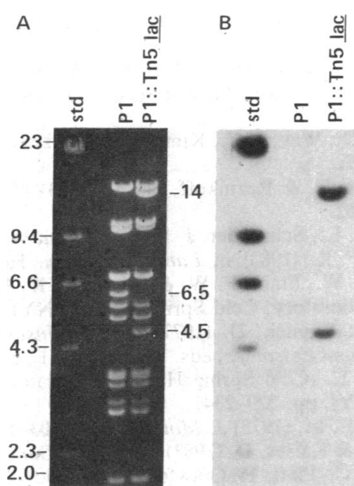

FIG. 3. Restriction (*A*) and Southern blot hybridization (*B*) analysis of P1::Tn*5 lac*. (*A*) DNA was digested with restriction enzyme, electrophoresed on a 0.5% agarose gel containing ethidium bromide, and photographed with UV illumination. Size standards (std) were *Hin*dIII-digested λ, and both P1 and P1::Tn*5 lac* were digested with *Eco*RI. Numbers represent kbp. (*B*) Autoradiogram of a Southern blot (27) of the same gel as in *A* probed with a mixture of λ and pLRKΔ211 DNA ^{32}P-labeled by nick-translation (10).

Introduction of Tn*5 lac* into *Myxococcus*. When P1::Tn*5 lac* infected *M. xanthus* strain DK101, Km^r transductants were obtained at a frequency of 72 ± 3 per 200 μl of 25-fold-concentrated phage stock. Since insertion of Tn*5 lac* increases the genome length of P1 by ≈12%, the DNA of phage particles will lack terminal redundancy and consequently will be unable to recircularize upon infection of *E. coli*. Such particles are not expected to form plaques. Under our conditions, thermal induction of P1 *clr100* lysogens generally yields ≈5 × 10^9 plaque-forming phage per ml. Assuming the P1::Tn*5 lac* lysate contains this concentration of phage particles, the transduction frequency of P1::Tn*5 lac* into *Myxococcus* is estimated to be 3 × 10^{-9} Km^r transductants per phage particle.

To verify that the Km^r tranductants result from transposition of Tn*5 lac* to the *Myxococcus* chromosome, the experiment shown in Fig. 4 was carried out. DNA from the DK101 parent strain forms no hybrids with a Tn*5 lac* probe, while the *Eco*RI digests of nine independent Km^r transductants each have two hybridizable fragments, as expected, since there is one *Eco*RI site in Tn*5 lac*. No two tranductants display the same set of fragments, showing that Tn*5 lac* has inserted at a different position in the *Myxococcus* chromosome in each transductant. Furthermore, comparing the sum of fragment lengths for individual transductants indicates that Tn*5 lac* had transposed to at least seven different regions of *Myxococcus* DNA. No hybridization was observed when P1 DNA was used as the labeled probe (data not shown).

Individual transductants express different levels of β-galactosidase as measured by colony color on CTT X-Gal, as if Tn*5 lac* had fused β-galactosidase expression to different *Myxococcus* promoters. The stability of Tn*5 lac* and its β-galactosidase expression were tested. Four transductants that were expressing β-galactosidase and two that were not were grown in the absence of kanamycin for 36 generations, plated on CTT, and 100 colonies for each were placed on CTT plates with Km and with X-Gal. All 100 colonies grew on the CTT Km plate and were the same color as originally

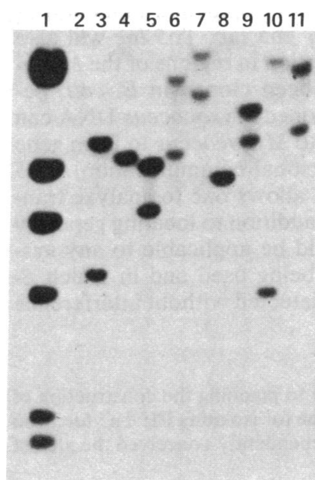

FIG. 4. Location of Tn*5 lac* in *Myxococcus* DNA. Chromosomal DNA was isolated (28), digested with *Eco*RI, electrophoresed on a 0.5% agarose gel, and Southern blot hybridization was carried out with a mixture of λ and pLRKΔ211 DNA as the ^{32}P-labeled probe. Lanes: 1, *Hin*dIII-digested λ; 2, DK101; 3–11, nine independent Km^r strains generated by infection of DK101 with P1::Tn*5 lac*.

determined on CTT X-Gal for each strain tested.

It was noted that *Myxococcus* colonies expressing a high level of β-galactosidase from Tn*5 lac* grew more slowly on CTT X-Gal plates than those expressing little or no β-galactosidase, and this effect was verified in liquid cultures supplemented with X-Gal (data not shown). Inclusion of X-Gal (50 μg/ml) in CTT plates prevents growth of *Myxococcus* strains expressing β-galactosidase from Tn*5 lac* but does not prevent growth of nonexpressing strains when cells are plated for single colonies (D. Hodgson, personal communication).

DISCUSSION

A transposable promoter probe, Tn*5 lac*, was constructed by inserting a promoterless *trp–lac* fusion fragment near the left end of Tn*5*. When Tn*5 lac* inserts in a transcription unit in the correct orientation for the *trp–lac* fusion segment to be transcribed, a polycistronic messenger RNA is made. Stop codons in all three reading frames in the first 80 bp of the *trp–lac* fusion segment (29) prevent translation in the wrong reading frame of *trpA* and *lacZYA*, which retain their normal translation start signals. Thus, Tn*5 lac* is designed to make only transcriptional fusions to promoters outside Tn*5*. While this work was in progress, a modified Tn*5* whose kanamycin resistance depends on outside promoters was described and was used to make operon fusions in *Caulobacter* (30).

We obtained an insertion of Tn*5 lac* in P1 that maps in the *Eco*RI fragment immediately downstream of the site at which P1 begins packaging its DNA into phage heads (31). This is the optimal position to ensure that almost every phage particle will contain Tn*5 lac*. This P1::Tn*5 lac* transduces *Myxococcus* to kanamycin resistance at ≈6% the frequency observed for P1::Tn*5* (5), consistent with the transposition frequency of Tn*5 lac* relative to Tn*5* in *E. coli*. Southern hybridization of 9 Km^r transductants revealed that all had single insertions of Tn*5 lac* at different positions in the *Myxococcus* chromosome, and this pattern has been extended by tests of 20 other Km^r transductants (unpublished data). Because of the wide host range of P1 (32) and Tn*5* (33), P1::Tn*5 lac* should be useful in a wide variety of bacteria.

By isolating large numbers of Tn*5 lac* insertions in the *Myxococcus* chromosome and screening them for β-galactosidase expression under growth and developmental conditions, we hope to identify genes that alter their expression during development. One way to study the regulation of developmentally induced genes is to devise a selection for mutations altering that regulation. We find that X-Gal is toxic to *Myxococcus* when it is cleaved by a strain expressing β-galactosidase, and it may be possible to select *cis*- and *trans*-acting mutations that eliminate developmental induction of β-galactosidase expression from Tn*5 lac*. Tn*5 lac* will also be useful for analysis of transcription in regions of the *Myxococcus* chromosome that have been cloned in *E. coli*, because insertions of Tn*5 lac* in cloned *Myxococcus* DNA can be obtained and reintroduced into *Myxococcus* to form gene replacements (K. Stephens, personal communication). Tn*5 lac* mutagenesis of cloned DNA allows one to analyze transcription in the cloned region in addition to locating genes by insertional mutation. This should be applicable to any system in which Tn*5* is currently being used and in which β-galactosidase can be made and detected without interference from endogenous sources.

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