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Genetics

Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *In vivo* probe for transcriptional control sequences

(gene regulation/ampicillin resistance/Tn3 transposon/*ara, mal, trp*/plasmids)

MALCOLM J. CASADABAN AND STANLEY N. COHEN

Departments of Genetics and Medicine, Stanford University, Stanford, California 94305

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ABSTRACT The lactose structural genes, without the lactose promoter, have been incorporated into the bacteriophage Mu genome to form a Mu-*lac* specialized transducing phage. This phage also carries a gene encoding resistance to ampicillin (Ap) [Mu(Ap, *lac*)]. After infection and upon establishment of lysogeny, the Mu(Ap, *lac*) genome can integrate into apparently random sites in the *Escherichia coli* chromosome. When integration occurs within a gene in the orientation of its transcription, the lactose structural genes are so situated that they become expressed solely from the promoter of that gene. Thus, expression of the lactose genes of Mu(Ap, *lac*) can be used as an assay for transcription of that gene and for functional and mutational studies of gene regulation.

In the study of gene regulation, it frequently is useful to join the promoter and controlling elements of one gene to the structural part of another well-characterized gene whose product is easy to assay. This type of gene fusion is especially useful for studying the regulation of genes (or operons) whose products are difficult to assay or whose products are not even known (1-3).

For this reason we have been developing rapid and simple techniques for joining the lactose (*lac*) structural genes to different promoters in *Escherichia coli*. The *lac* genes are especially convenient for this because of the well-developed biochemical and genetic methods available (4, 5). These methods include sensitive enzyme assays and convenient mutant selection procedures.

Previous methods for fusing the *lac* structural genes to other promoters have required several genetic steps (6-11). These steps have included translocation of the *lac* genes to be near the promoter of interest and subsequent isolation of the precise fusion desired. Here we describe an *in vivo* procedure for translocating and fusing the *lac* genes to a random promoter in one step. For this, we have used the integration properties of the bacteriophage Mu genome (for reviews of Mu, see refs. 12 and 13).

The bacteriophage Mu genome can integrate into the host bacterial chromosome at apparently random sites; however, the Mu genome itself integrates in a unique way. By incorporating the *lac* genes inside of the Mu genome, the *lac* genes can be directly transposed as a part of the Mu-*lac* genome to random sites. Furthermore, if the *lac* genes are located sufficiently near one end of the Mu genome, then transcription through the site of insertion could proceed directly into the Mu-*lac* to express the *lac* genes. No deletion or other genetic event would be needed to complete the final fusion. We report here the construction of such a Mu-*lac* specialized transducing phage and the use of this phage as a probe for transcriptional control signals.

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MATERIALS AND METHODS

Media. The media have been described (14). Cells were usually grown in LB liquid medium and plated on lactose/MacConkey agar solid medium in petri plates. On lactose/MacConkey plates, the amount of red color of a bacterial colony corresponds to the amount of lactose fermentation (Lac$^+$ phenotype) which is due to the amount of expression of the *lacZ* (for β-galactosidase) and *lacY* (lactose permease) genes. Ampicillin (Ap) was used at 25 μg/ml, kanamycin (Km) at 20 μg/ml, and streptomycin (Sm) for chromosomal *strA*^{R} at 100 μg/ml.

Bacteria and Phage Manipulations. Strains are listed in Table 1. For all experiments, the temperature-sensitive Mu repressor mutation (Mu *cts*), was used. Lysogens of Mu *cts* were grown between 28 and 32°C. Mu *cts* cultures were thermoinduced by growing in LB medium to early exponential phase ($\approx 1 \times 10^8$ cells per ml) at 28-32°C, shifted to 41.5-44.5°C for 15-25 min, and incubated at 37°C for about 50 min until lysis. Chloroform (1%, vol/vol) was added, the lysate was stirred, and, after 5 min, was stirred again and centrifuged to remove cell particles. Mu lysates can lose infectivity rapidly and so were usually used within a few days. Titer was about 10^{10} plaques per ml and, for Mu-Ap, about 10^7 Ap^R transductants per ml.

For Mu infection, recipient cells were grown in LB medium to early stationary phase ($\approx 10^9$ cells per ml), made 10 mM in $MgSO_4$ and 5 mM in $CaCl_2$, and absorbed for 20 min at 28-30°C with Mu lysates that had been diluted to less than 1 phage per cell. To allow expression of an Ap^R gene carried by Mu, the infected cells were diluted 1:10 with LB medium and grown at 28-30°C for 30 min (a time shorter than that needed for lysis to occur after lytic growth). Ap^R transductants were selected either by plating on Ap plates or by diluting 1:10 and growing in LB/Ap medium.

RESULTS

Isolation of the Mu-*lac* Phage. The *lac* genes were first fortuitously placed near one end (the "S" end) of Mu by a spontaneous deletion event. This deletion, ΔF1, was isolated from a recombinant Mu insertion located between the *ara* promoter and the *lac* structural genes (10) (Fig. 1*a*). ΔF1 was selected as the loss of Mu sequences that block transcription from the arabinose-induced *araI* promoter from reaching and expressing the *lac* structural genes. The fact that a terminal piece of the bacteriophage Mu genome remains between *ara* and *lac* in the F1 fusion was inferred from restriction endonuclease mapping (unpublished data). This terminal Mu genomic DNA segment left by ΔF1 apparently does not contain any strong transcription termination or polarity sequences because arabinose-induced transcription can proceed across it to express *lac*.

Abbreviations: Ap, ampicillin; Km, kanamycin; Sm, streptomycin; ^{R}, resistant.

Table 1. Strains and plasmids

	Genotype	Source
EC601.9	F′ ts 114, *lac*I^s::Mu *c* ts, *lac* $P^+O^+Z^+Y^+A^+/F^-$ Δ(*lac*IPOZYA) U169, *mel*B?, *glp*?, *thi*	Ref. 11
MAL103	F^-, Mu *c* ts d1(Ap^R, *lac*), Mu *c* ts, Δ(proAB, *lac*IPOZYA)XIII, *str*A	This work
MC4100	F^-, *ara*D139, Δ(*lac*IPOZYA)U169, *str*A, *thi*	Ref. 11
MC4143	MC4100 with *ara*B::Mu *c* ts	Ref. 11
pMC81	ColE1::Tn3(Ap^R):*Bam*I:(*ara*-Mu′-*trp*-*lac*)F1	Fig. 1*b*
pML21	ColE1-derived plasmid containing colicin E1 immunity and Km^R	Ref. 15

Genetic nomenclature is from ref. 16.

Mu DNA integration apparently requires both ends of the Mu genome. To arrange for the other end (the *c* end) of the Mu genome to be on the other side of the *lac* genes, the whole *ara*-Mu-*trp*-*lac* F1 fusion was first inserted into the *Bam* I site of the DNA transposition element Tn3 by *in vitro* DNA cloning (details to be given elsewhere) to form a Tn3-*ara*-Mu-*trp*-*lac* transposon (Tn3-81, Fig. 1*b*). Because the *Bam* I site of Tn3 is not essential for transposition, the entire Tn3-81 transposon could transpose into an intact Mu genome, placing ends of Mu on both sides of *lac* (Fig. 1 *c* and *d*) and allowing the replication and phage capsid packaging of the desired Mu-*lac* phage (Fig. 1 *d* and *e*).

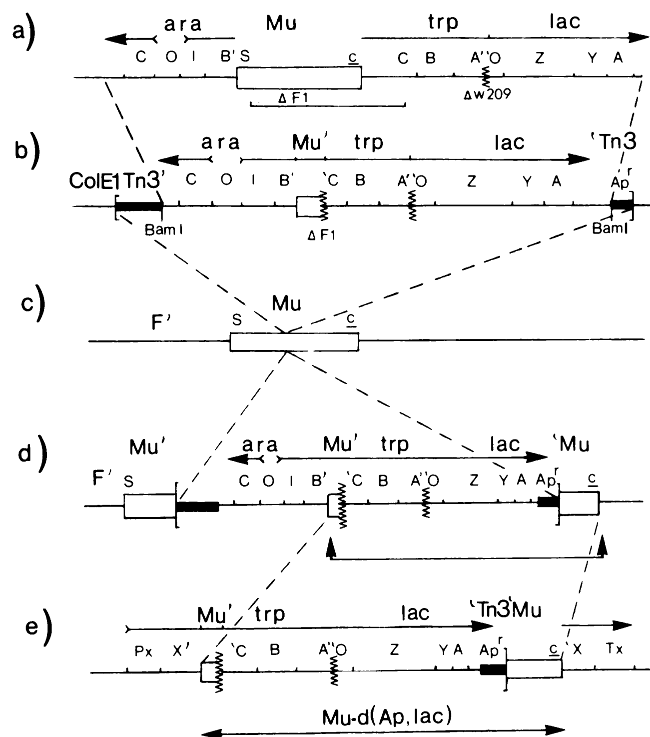

FIG. 1. Isolation of the Mu(Ap, *lac*) specialized transducing phage. (*a*) A recombinant Mu insertion (open box) positioned between the *araI* promoter and the *lac* structural genes, isolated as described (7). *c* and S represent terminal Mu genes. The F1 deletion removes Mu termination sequences, allowing transcription (overhead arrows) to proceed from the *araI* promoter into the *lac* structural genes. The original *lac* promoter had been removed and replaced by DNA from within the *trp* operon (8). A prime next to a genetic symbol indicates that it is deleted or interrupted on the side on which the prime is written. These genetic regions are not all drawn to scale; the Mu insertion in particular is much larger (37,000 nucleotides). (*b*) The pMC81 plasmid including the entire F1 *ara*-Mu′-*trp*-*lac* fusion inserted into the *Bam* I site of the Tn3 (thick lines) DNA transposition element to form Tn3-81. This map of the circular plasmid is arbitrarily opened and drawn linearly, as are the other plasmid maps in this figure. (*c*) The F′ episome of EC601.9 containing a Mu insertion. (*d*) Tn3-81 inserted by Tn3 transposition into F′ Mu. Although Tn3-81 can integrate anywhere in F or Mu sequences, and in either orientation, only the desired orientation and location is drawn. The vertical arrows indicate the two Mu ends used to form Mu(Ap, *lac*). These ends must be close enough together to allow packaging into Mu capsids. (*e*) Mu(Ap, *lac*) insertion in a gene X in the orientation that fuses transcription from the promoter Px to *lac*. Note that the insertion must be between the promoter and terminator (T) of the gene but it need not be within the structural gene.

To obtain the transposition of Tn3-81 into Mu, the pMC81 (Tn3-81) plasmid was placed by DNA transformation into EC601.9 which contains a Mu insertion on an episome (F′ Mu). In a rare EC601.9/pMC81 cell, Tn3-81 was expected to insert by transposition into the Mu genome in an appropriate orientation at an appropriate distance from the Mu ends. Such rare insertions of Tn3-81 (Ap^R) into Mu were enriched by selecting for Ap^R transfer to another cell by F′ Mu—by mating (at 32°C) EC601.9/pMC81(Ap^R) with MC1040(Sm^R)/pML21 (Km^R) and selecting exconjugants by overnight growth in medium containing Ap, Sm, and Km. (pMC21 was used in the recipient because it is incompatable with the pMC81 plasmid and thus would interfere with replication of any transferred pMC81 plasmid.) This exconjugant mixture was expected to consist of cells with Tn3-81 inserted into random sites of the F′ or Mu sequences. Assuming that only appropriate insertions of Tn3-81 into Mu could give rise to Mu(Ap, *lac*)phage (Fig. 1*d*), we thermoinduced the whole culture and used the resulting Mu lysate to transduce MC4100 to Ap^R. Ap^R was selected instead of Lac^+ because the Ap^R determinant was expected to be present on the resulting phage (Fig. 1*d*) and because many of the resulting Mu(Ap, *lac*)phage lysogens would not have promoters fused to *lac* and so would not be Lac^+.

Characterizing the Mu-d(Ap *lac*) Candidates. Twenty Ap^R transducants of MC4100 were obtained on lactose/MacConkey/Ap plates in the first experimental attempt. These showed different levels of *lac* expression, as expected if each was randomly fused to a different *E. coli* promoter. Eight transductants were purified by streaking to single colonies; only one was found to be able to release Mu plaque-forming phage. This one and three of the non-Mu-releasers were tested for the release of Mu(Ap, *lac*) transducing phage. To complement possibly defective Mu-Ap phages, a Mu prophage was introduced into these four by mating with strain EC601.9(F′ Mu). After mating for 100 min, the EC601.9 donor strain was killed by growing the cultures overnight in the presence of Sm; these cultures were thermoinduced to make Mu lysates.

All four lysates yielded Ap^R transductants with strain MC4100 at a high frequency ($\approx 10^{-3}$ Ap^R transductants per plaque-forming unit at limiting phage dilutions). For the three original non-Mu-releasing isolates, the expected wide range of *lac* expression was observed for the Ap^R colonies on lactose/MacConkey/Ap indicator plates. Of 10 Ap^R transductants tested from each of these three lysates, none released Mu phage. Thus, these clones appeared to carry defective Mu phage that could be complemented by a wild-type Mu prophage. For the fourth lysate that was derived from the Mu-releasing candidate, the Ap^R transductants all showed identical levels of *lac* expression ("$Lac^{\pm}$"). This candidate was not studied further.

To determine whether these Mu(Ap, *lac*) candidate phages could integrate their genomes into different sites around the chromosome like the parent bacteriophage Mu, we tested for the formation of auxotrophs. For bacteriophage Mu it is known that 1–2% of insertions result in auxotrophy. Twenty-five Ap^R Lac^+ transductants of MC4100 for each of two lysates were selected on lactose/MacConkey/Ap plates and checked for growth on minimal M63/glucose plates. For one lysate, 1 of the 25 transductants did not grow on the minimal plates but could grow if supplemented with isoleucine and valine. For the other, two did not grow on the minimal plates. One of these could also be supplemented with isoleucine and valine, and the other with glycine or serine. Thus, a total of 3 of 50 Lac^+ lysogens had insertions in essential biosynthetic genes.

For convenience in making Mu(Ap, *lac*) lysates, a strain lysogenic for both Mu 1(Ap, *lac*) and a helper Mu *c*ts was constructed. Because no Mu(Ap, *lac*) lysogen that contained a helper Mu *c*ts insertion was found, one was introduced by mating a Mu 1(Ap, *lac*) lysogen with an Hfr Mu *c*ts to form strain MAL103 (Table 1).

Subsequently we found that the Mu(Ap, *lac*) phage could lysogenize a strain already lysogenized for Mu *c*ts at 1/100th the frequency ($\approx 10^{-5}$ Ap^R transductants per plaque-forming unit). Twenty-four Ap^R transductants of a Mu *c*ts lysogenic strain (MC1040) were tested and found to retain the original Mu *c*ts because they could release Mu plaque-forming phage. These Ap^R transductants, like those of a nonlysogenic strain, showed a wide range of Lac phenotypes. Thus, the presence of a previously inserted Mu did not completely abolish Mu(Ap, *lac*) integration and apparently did not bias the site of Mu(Ap, *lac*) insertion.

The Mu(Ap, *lac*) genomes apparently are capable of integrating into the chromosome without a helper Mu phage. Ap^R transductants followed one-particle kinetics because dilution of the phage lysate gave a linear reduction in Ap^R transductants. Thus, a helper Mu phage need not infect the same cell as the Mu(Ap, *lac*) in order to establish an insertion. This is consistent with the finding that none of 50 Ap^R transductants checked contained a second Mu *c*ts insertion.

This proficiency of integration for the Mu(Ap, *lac*) phages implies that they contain the Mu A and B genes which are essential for Mu integration (12, 13). This is consistent with the observation that Mu(Ap, *lac*) lysogens are temperature sensitive for growth, because temperature sensitivity for the parental Mu *c*ts requires the presence of the early Mu gene region.

To show that the *lac* genes were actually fused to chromosome promoters, we examined insertions in the *ara* and *mal* genes. Gene expression from some *ara* and *mal* promoters is easily checked because it is induced to a high level by L-arabinose and maltose, respectively. Mu(Ap, *lac*) lysogenic strains having insertions in *araA*, *-B*, and *-C* were selected with strain MC4100 by using the L-arabinose-sensitive property of the $araD^-$ mutation (10, 17). Of 50 arabinose-resistant Ap^R lysogens selected on arabinose/MacConkey plates, 40% had *lac* expression induced by arabinose (Table 2); on lactose/MacConkey plates they were Lac^- and on lactose/MacConkey/arabinose plates they were Lac^+. Forty percent is the frequency expected for this phenotype because only insertions in *araA* and *araB*, but not *araC*, can have *lac* expressed from the arabinose-inducible *araI* promoter, and then only when inserted in the orientation of transcription. *araC* expression is not inducible by arabinose (1).

Insertions in maltose genes were also selected as mal^- (red) colonies on maltose/tetrazolium/Ap plates. Some of these were also found to have *lac* expression induced by maltose (Table 2) (18).

Table 2. β-Galactosidase assays for Mu-dl(Ap, *lac*) insertions into *ara* and *mal*

Insertion	β-Galactosidase, units			Ratio of induction
	No addition	With arabinose	With maltose	
Ara1	29	530		18
Ara2	4.2	380		90
Mal1	21		180	8.6

Bacteria were grown in minimal M63/0.2% glycerol medium and assayed as described (14). L-Arabinose and L-maltose were added to 0.1%.

DISCUSSION

We have described the construction of a Mu(Ap, *lac*) phage which may be used to rapidly fuse the lactose structural genes to new promoters in any bacterial species into which Mu can be introduced. The degree to which the lactose genes are expressed depends on the amount of transcription that is not attenuated before reaching the *lac* genes. The *lac* expression is also limited by the efficiency of initiation of *lac* translation from the fused mRNA. Thus, the level of *lac* expression from a promoter is only a minimal measure of the transcription initiated at that promoter, and different levels of *lac* expression can result from different fusions to the same promoter (6–9) (Table 2). However, any transcriptional regulation of the gene whose promoter is fused to *lac* should apply to expression of the fused lactose genes if the transcriptional control sites are intact (1–3, 6–11).

Genes to be fused to *lac* need not originate in the Mu-sensitive bacteria but might be introduced by appropriate DNA cloning techniques. We have been able, for example, to isolate Mu insertions into the medium-copy-number pSC101 plasmid cloning vector but not into the very-high-copy-number plasmids derived from ColE1 or p15A (unpublished results).

The ability to isolate quickly large numbers of lactose fusions to random promoters permits the use of new approaches to the study of genetic control mechanisms. For example, by using Mu(Ap, *lac*) insertions, genes can be identified solely by the criteria of how they are regulated, without a knowledge of their mutant phenotypes or chromosome position. D. Kolodrubetz and R. Schleif (personal communication) have already used this approach with Mu(Ap, *lac*) to identify the *E. coli* K-12 L-arabinose transport genes solely by their inducibility by L-arabinose. It would have been difficult to identify these genes by their mutant phenotypes because there are two L-arabinose transport systems, and mutations in genes for either one would not fully abolish L-arabinose active transport. Similarly, promoters present on the host chromosome, on coexisting plasmids, or on cloned segments of introduced DNA can be identified and rapidly grouped according to their relative strength, as indicated by the intensity of expression of the *lac* genes on Mu(Ap, *lac*).

We have also recently constructed a λ-*lac* phage, λp540-773, similar to Mu(Ap, *lac*) (unpublished results). This phage has the lactose structural genes, without the lactose promoter, adjacent to the λ PP′ attachment site such that the lactose genes can become expressed from adjacent bacterial promoters upon integration into the bacterial chromosome. However, this λ phage is not as useful as the Mu phage because λ integrates into the chromosome at preferred sites and because two λs can integrate together, thereby joining λ promoters to the lactose genes.

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