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NOTES

Isolation of an *aroF-lac* Plasmid by Recombination In Vivo

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An *aroF-lac* operon fusion was transferred from a λ *aroF-lac* prophage onto a plasmid carrying a 'Mu *cts* *trp-lac* fusion by recombination in vivo.

The general procedure devised by Casadaban (2) for the construction of operon fusions and the isolation of λ transducing phage carrying such fusions has been widely and successfully applied to various regulatory systems in *Escherichia coli* K-12. We have applied this procedure to the construction of fusions of the *lac* operon to the regulatory region of the *aroF-tyrA* operon, an operon involved in the biosynthesis of the aromatic amino acids and regulated by the gene *tyrR*$^+$ (11). Although *aroF-lac* λp1(209) fusion strains were obtained, all λ *aroF-lac* transducing phage isolated from these strains were found to be unstable. During the lytic cycle of growth the poorly growing λ *aroF-lac* transducing phage were rapidly overgrown by large plaque-forming Lac$^-$ derivatives (3). We had intended to use the DNA of such a transducing phage as a template for an in vitro transcription-translation assay of the *tyrR*$^+$ gene product and for cloning the fusion into a plasmid vector by in vitro methods. However, the instability of these phage prevented the purification of a homogeneous DNA preparation, thereby making the in vitro approach impractical. This paper describes the construction by recombination in vivo of a plasmid carrying an *aroF-lac* fusion.

Figure 1 shows that some transducing phage formed from an *aroF-lac* λp1(209) fusion strain might incorporate both the fusion and the region of Mu DNA at the distal end of the prophage. The genome of such transducing phage would be analogous in structure to λp1(209) (7) but with the *aroF* regulatory region interposed between the *trp-lac* DNA and the 'Mu *cts* DNA. Because λ *aroF-lac* transducing phage are stable in the lysogenic state, they can be isolated as prophages by transducing an appropriate *tyrR* Δ*lac* recipient strain and selecting for Lac$^+$ transductants (3). Recombination between a λ 'Mu *cts* *aroF-lac* prophage and a plasmid carrying the 'Mu *cts* *trp-lac* region of λp1(209) was expected to transfer the *aroF-lac* fusion onto the plasmid (Fig. 1). For this recombination to occur it was essential, when isolating a λ 'Mu *cts* *aroF-lac* lysogen, to maintain the continuity of the Mu, *aroF*, and *lac* regions of the prophage. Integration of the prophage into the chromosome via homology with the *aroF* region would result in the fusion no longer being flanked by the 'Mu *cts* DNA on the "upstream" side. If, however, integration occurred via homology with λ DNA, there would be no disruption of the 'Mu *cts* *aroF-lac* region. Therefore, the *tyrR* Δ*lac* λ *imm*^{434} lysogen JP2978 (3) was used as the recipient. Integration of the incoming prophage into the chromosome of this strain was expected to occur via homology with the resident, heteroimmune λ *imm*^{434} prophage.

JP2978 was transduced with a lysate prepared by UV induction of the *aroF-lac* λp1(209) fusion strain JP3110 (3). Genetic procedures have been described previously (3). Lac$^+$ lysogens were selected, and those in which the pro-

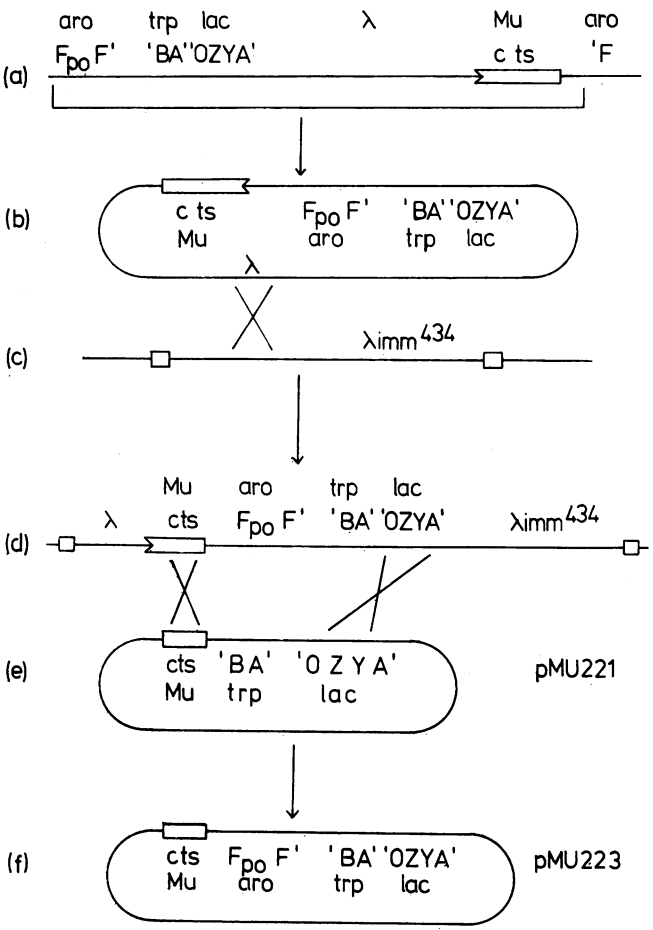

FIG. 1. Isolation of an *aroF-lac* plasmid. Induction of an *aroF-lac* λp1(209) fusion strain (a) with UV light resulted in the formation of a λ 'Mu *cts* *aroF-lac* transducing phage (b). Integration of this phage into the chromosome via homology with a λ *imm*^{434} prophage (c) resulted in the formation of a λ 'Mu *cts* *aroF-lac* lysogen (d). Recombination between the λ 'Mu *cts* *aroF-lac* prophage and pMU221, a plasmid carrying the 'Mu *cts* *trp-lac* region of λp1(209) (e), resulted in the formation of pMU223, an *aroF-lac* plasmid (f).

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TABLE 1. β-Galactosidase activities of strains with plasmids carrying the *aroF-lac* fusion[a]

Plasmid(s) present	*tyrR* allele present	β-Galactosidase activity (U)	
		MM	AA
pMU223	+	4,650	2,770
pMU224	+	4,680	2,660
pMU225	+	547	180
pMU225	366	2,530	2,360
pMU225 pMU355	+ (multicopy)	407	70

[a] Strains were grown in minimal medium 56/2 (9) (MM) and MM containing 10^{-3} M tyrosine, 10^{-3} M phenylalanine, and 10^{-3} M tryptophan (AA). Medium for strains carrying pMU225 included 2 μg of trimethoprim per ml. β-Galactosidase activities were determined by the method of Miller (8). pMU355 is a derivative of pBR322 carrying the *tyrR*$^+$ allele (5).

phage carried the 'Mu *c*ts region were identified by their immunity to Mu phage at 30°C and sensitivity at 42°C. One such λ 'Mu *c*ts *aroF-lac* lysogen, JP3458, was isolated. The expression of β-galactosidase activity in this strain was regulated by *tyrR*$^+$ similar to that in the original fusion strain, JP3110 (data not shown). JP3458 was used in an interrupted mating with a Δ*lac* Hfr strain, and it was demonstrated that the prophage was integrated via homology with the λ *imm*^{434} prophage and not with the *aroF* locus (data not shown).

The next step of this procedure was to clone the 'Mu *c*ts *trp-lac* region of λp1(209) into the multicopy plasmid pBR322 in vitro. This entire region of λp1(209), including the region of Mu DNA coding for the temperature-sensitive Mu repressor, lies on a 4.7-megadalton (Md) *Pst*I restriction endonuclease fragment (data not shown). *Pst*I fragments of λp1(209) were cloned into pBR322 and transformed into a *tyrR*$^+$ Δ*lac* recipient, JP2869 (3), using the methods of Davis et al. (5). By selecting for transformants immune to Mu *c*ts at 30°C and resistant to tetracycline, a plasmid, pMU221, carrying the 4.7-Md fragment was isolated. The strain carrying pMU221 expressed a low level of β-galactosidase (5 U), although there are no known promoters transcribing the *lac* genes.

The final step required the recombination between the λ 'Mu *c*ts *aroF-lac* prophage and pMU221 to transfer the fusion onto the plasmid. For this, *tyrR*$^+$ was introduced into JP3458 by conjugation with Hfr strain CA7027 (3) to form JP3459. JP3459 was then transformed with DNA of pMU221, selecting for transformants resistant to tetracycline, to form JP3460. JP3460 expressed only 25 U of β-galactosidase due to the presence of *tyrR*$^+$. Derivatives of JP3460 in which the desired recombination had occurred and which consequently carried many copies of the *aroF-lac* fusion were expected to be more strongly Lac$^+$. A culture of JP3460 was enriched for more strongly Lac$^+$ cells by growth in minimal medium containing lactose as the sole carbon source plus 10^{-3} M tyrosine to repress the expression of the *aroF-lac* fusion. JP2869 was transformed with plasmid DNA prepared from this enriched culture, and transformants were selected on lactose minimal agar containing tetracycline. About 1% of the tetracycline-resistant transformants obtained were strongly Lac$^+$, and in three of six tested the β-galactosidase activity was partly repressed in the presence of the aromatic amino acids. The plasmid in one of these strains (Table 1) was designated pMU223 and was provisionally assumed to carry the *aroF-lac* fusion.

The physical maps of both pMU221 and pMU223 were similar in many respects as both carried the 'Mu *c*ts and *lac* DNA (Fig. 2). The relative positions of the cleavage sites upstream of the *lacZ* gene in pMU223 corresponded closely to the physical map of the chromosomal region upstream of the *aroF-tyrA* operon (10; B. Davidson, personal communication). The 1.5-Md *Hin*dIII fragment, which includes all chromosomal DNA beyond the *Hin*dIII site immediately upstream of *lacZ*$^+$, was excised from pMU223 to form pMU224 (Fig. 2e). This plasmid expressed the same level of β-galactosidase activity as pMU223 (Table 1), demonstrating that the entire *aroF-lac* fusion was situated on the 4.5-Md *Hin*dIII fragment of pMU223. Also, since all but the 0.54-Md *Hin*dIII/*Hpa*I fragment of pMU224 is also on pMU221, then this fragment must carry the *aroF* regulatory region (Fig. 2).

The partial repression of the *lac* genes on pMU223 in the *tyrR*$^+$ strain could have been attributed to titration of repressor molecules by the many copies of the *aroF* operator. However, the ability of repressor to regulate the transcription of the *aroF-lac* fusion on pMU223, and hence its usefulness as a template for assaying repressor activity, remained in some doubt. This was resolved by cloning the

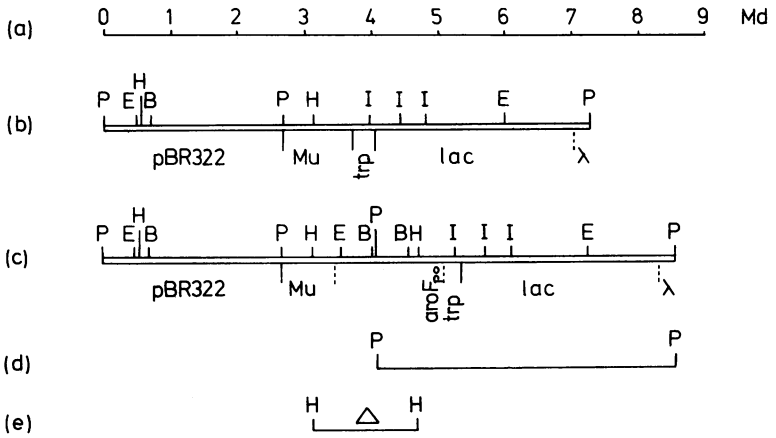

FIG. 2. Physical maps of pMU221 and pMU223. (a) Scale in megadaltons; (b) pMU221; (c) pMU223. The *Pst*I fragment of pMU223 cloned into pREG151 to form pMU225 is indicated in (d), and the *Hin*dIII fragment excised from pMU223 to form pMU224 is indicated in (e). Both pMU221 and pMU223 have been drawn as linear molecules for convenience. The cleavage sites for the restriction enzymes *Pst*I (P), *Eco*RI (E), *Hin*dIII (H), *Bam*HI (B), and *Hpa*I (I) and the derivations of the various regions of each plasmid are indicated above and below each representation, respectively. The junctions between these regions are indicated by dotted lines where the precise position of the junction is not known.

4.6-Md *PstI* fragment (Fig. 2d) of pMU223 into pREG151. pREG151 is derived from R388, a transferable drug resistance plasmid of the *incW* incompatibility group, which codes for resistance to trimethoprim and exists at one to two copies per cell (S. Falkow, personal communication). The β-galactosidase activities expressed by the low-copy-number *aroF-lac* plasmid pMU225 in JP2869 and its derivatives are shown in Table 1. The derepression of the *lac* genes caused by the *tyrR366* allele and the additional repression in the presence of the multicopy $tyrR^+$ plasmid pMU355 (4) show that the expression of the *lac* genes was regulated by $tyrR^+$.

This procedure can be used as a general method for cloning operon fusions from unstable transducing phage constructed by Casadaban's method. Although no other reports of such instability have been published, it seems unlikely that this should be an isolated occurrence. One possible reason for the instability of λ *aroF-lac* transducing phage could be that the size of their genomes prevented them from being packaged efficiently. pMU223 was 1.3 Md larger than pMU221, indicating that the λ 'Mu *cts aroF-lac* prophage from which it was derived was 1.3 Md (or 4% of the wild-type λ genome [1]) larger than λp1(209). However, since λp1(209) is only 97% of the size of λ wild type (7) and since up to 105% of the λ genome can be packaged efficiently (6), then the genome of the λ 'Mu *cts aroF-lac* prophage (101%) is within the packageable limits.

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