SHORT COMMUNICATION

Construction and Characterization of *guaB*–*lacZ* Fusions in *Escherichia coli* K12

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GuaB–*lacZ* fusion strains of *Escherichia coli* K12 have been constructed using the Casadaban (1976) gene-fusion technique. The major modification to the procedure was the removal of the guanine requirement of Mu-induced *guaA* auxotrophs by introduction of a ColEI–*gua*⁺ plasmid prior to selection of the fusion. Conversion to prototrophy was necessary to overcome problems associated with repression of the *gua* operon by guanine added to selection media. Induction of the lysogenic fusion strains yielded plaque-forming λ transducing phages that carried the *lac* genes and either the complete *guaB* gene and the *gua* promoter or only a distal portion of *guaB*.

INTRODUCTION

The *gua* operon of *Escherichia coli* comprises two structural genes, *guaB* and *guaA*, coding for IMP dehydrogenase and GMP synthetase, respectively. These enzymes are involved in the final two reactions of GMP biosynthesis. The genes are co-ordinately regulated and are arranged promoter–*guaB*–*guaA* (Lambden & Drabble, 1973; Shimada *et al.*, 1976). The operon is controlled both by repression by guanine and induction by adenine nucleotides so that a 200-fold variation in operon expression can be detected (Lambden & Drabble, 1973; Mehra & Drabble, 1981). A useful general technique for studying the regulation of operons has been developed by Casadaban (1976). This involves joining the lactose (*lac*) structural genes to the promoter and controlling elements of the operon under investigation using a λ Mu *plac* hybrid phage. Using this technique we have isolated strains containing fusions that place the *lac* genes under the control of the *gua* operon. From these strains specialized λ transducing phages carrying *gua*–*lac* fusions have been isolated.

METHODS

Media. Defined salts medium (minimal medium) was that of Vogel & Bonner (1956) containing glucose or lactose (final concentration 2 mg ml^{–1}) as carbon source. Minimal agar was prepared by adding 1.5% (w/v) Difco Bacto-agar. Guanine, xanthine and amino acids, when required, were added to media at a final concentration of 20 μ g ml^{–1} unless stated otherwise. 5-Bromo-4-chloro-3-indolyl- β -D-galactoside (X-gal; Sigma) was dissolved in *N,N*-dimethylformamide, and used at a concentration in media of 20 μ g ml^{–1}. Complex media used were tryptone broth (T-broth), T-agar, L-broth and L-agar (Schrenk & Weisberg, 1975). For plate overlays, soft T-agar (0.35% agar) or soft L-agar (0.6% agar) were used.

Mutagenesis with phage Mu. Phage Mucts62 (Howe, 1973) was spotted on to L-agar plates overlaid with soft L-agar seeded with strain X7029 [Δ (*lac*)X74]. After overnight incubation at 32 °C, bacteria were removed with a loop from the centre of areas of lysis and inoculated into minimal guanine medium for the selection of *guaA* auxotrophs (Gilbert & Drabble, 1980).

Determination of Mu orientation. The method has been described by Zeldis *et al.* (1973). Two Hfr donors were constructed using the F-prime strains EC601.9 and EC601.14 (Casadaban, 1976). The F[–] recipient was PC0147

(H865: *thi his tyrA trp lacY gal xyl mal tonA tsx lam rpsL phx*; Nijkamp & DeHaan, 1967). Recombinants (*tyrA*⁺ and *his*⁺) were selected after 30 min mating.

Isolation of λ p123(209) lysogens. λ p123(209) (Leathers *et al.*, 1979) lysogens of strain X7029 with Mu inserted in *guaA* were isolated by the method of Casadaban (1976) using phages λ b2c and λ h80cde19 (Shimada *et al.*, 1972) and λ gv (a virulent mutant).

Transfer of the plasmid pLC34-10. Overnight cultures of strains NS1063 and JA200(pLC34-10) (Clarke & Carbon, 1979) in L-broth were mixed in the proportion 10:1 and diluted 100-fold into fresh L-broth. After incubation overnight at 32 °C, bacteria were washed in 0.85% (w/v) NaCl and plated on to unsupplemented minimal medium to select *guaA* mutants that had acquired the pLC34-10 plasmid. The plasmid was transferred with a frequency of about 10⁻⁵.

Selection for *gua-lac* fusions. Strain NS1064 was grown overnight in L-broth. The bacteria were centrifuged and washed with 0.85% NaCl. Samples (0.1 ml) were plated on to lactose minimal plates and incubated overnight at 42 °C then overnight at 37 °C.

Transduction. The P1cmlcr100 bacteriophage was used for generalized transduction according to the method of Rosner (1972). Specialized transduction with λ phages was performed as described by Schrenk & Weisberg (1975).

Preparation of phage lysates. Lysates of phage P1 were prepared by the soft-overlay method (Lambden & Drabble, 1973). λ lysates were prepared from *gua-lac* fusion strains by u.v.-irradiation. Bacteria (5 ml) from overnight cultures in T-broth were washed, resuspended in 10 ml 0.85% NaCl, then irradiated for 2 min in an open Petri dish 500 mm from a 30 W Hanovia 'Chromatolite' (254 nm emission). The bacteria were centrifuged, resuspended in 10 ml T-broth and incubated with shaking at 37 °C for 2 h. After the addition of chloroform (0.1 ml), cell debris was removed by centrifugation and MgSO₄ was added to a final concentration of 10 mM. λ lysates were prepared from single plaques by lytic growth on strain X7029 [Δ (*lac*)X74]. A single plaque was inoculated into 2 ml of exponentially growing X7029 in T-broth in the presence of mitomycin C (1 μ g ml⁻¹). After 6 h a few drops of chloroform were added, cell debris was removed by centrifugation and MgSO₄ was added to 10 mM.

Enzyme assays. IMP dehydrogenase [IMP:NAD⁺ oxidoreductase; EC 1.2.1.14] and GMP synthetase [xanthosine-5'-phosphate:ammonia ligase (AMP-forming); EC 6.3.4.1] were assayed in cell-free extracts (Lambden & Drabble, 1973) by the methods of Gilbert *et al.* (1979) and Lambden & Drabble (1973), respectively. The β -galactosidase assay was described by Nicolaidis & Drabble (1979). One unit of β -galactosidase hydrolyses 1 μ mol *o*-nitrophenyl β -D-galactoside min⁻¹ at 37 °C. Protein was determined by the Lowry method using bovine serum albumin as standard.

RESULTS

Isolation of Mu-induced *guaA* auxotrophs. Strain X7029 [Δ (*lac*)X74] was mutagenized with Mu as described in Methods and guanine-requiring mutants were selected by replica plating after penicillin screening. Five *guaA* mutants were identified by their specific requirement for guanine; one of these (NS1057) was chosen for further investigation. GMP synthetase was not detectable in cell-free extracts of NS1057 and growth of this mutant with a low concentration (4 μ g ml⁻¹) of guanine in the medium resulted in derepression of IMP dehydrogenase indicating a functional *guaB* gene. Transduction with phage P1cmlcr100 confirmed that the mutation in NS1057 was Mu-induced. Phage P1 grown on strain W3110 (*guaA*⁺) transduced NS1057 back to prototrophy. All of 25 prototrophic transductants tested acquired Mu-sensitivity and the mutant was therefore presumed to have Mu inserted into *guaA*. The five Mu insertions into *guaA* were found to have the 'negative' orientation (Zeldis *et al.*, 1973), that is, gene *s* of the prophage precedes gene *c* when reading the chromosome map in the clockwise direction.

Isolation of λ p123(209) lysogens. The *gua* operon is transcribed in the anticlockwise direction on the chromosome of *E. coli* (Vales *et al.*, 1979). Strain NS1057 with Mu inserted into *guaA* in the negative orientation was lysogenized with λ p123(209) in order to place the *lac* genes in the same transcriptional direction as the *gua* operon. Lysogens (e.g. NS1063) were selected by their resistance to λ b2c. They were also resistant to λ h80cde19, but sensitive to λ gv. They had Lac⁻ phenotype.

Selection of strains with *gua-lac* fusions. Strain NS1063 with Mu, *lac* and λ prophage inserted into *guaA* is an auxotroph and requires guanine for growth on defined medium. Any

Fig. 1. Differential synthesis of β -galactosidase in cultures of strain NS1066 grown in minimal medium containing glucose (2 mg ml^{-1}) and guanine at a concentration of $40 \text{ } \mu\text{g ml}^{-1}$ (●), $4 \text{ } \mu\text{g ml}^{-1}$ (○), and $4 \text{ } \mu\text{g ml}^{-1}$ in the presence of 8-azaguanine ($100 \text{ } \mu\text{g ml}^{-1}$) (△). The arrows indicate the re-addition of guanine to a final concentration of $40 \text{ } \mu\text{g ml}^{-1}$ to guanine-depleted cultures.

fusion that places the *lac* genes under the control of the *gua* promoter region may be unable to utilize lactose as the carbon source because the fused operon would be repressed by the necessary guanine supplement in the medium. Attempts to isolate *gua-lac* fusions by supplying low (and possibly non-repressing) concentrations of guanine (down to $0.1 \text{ } \mu\text{g ml}^{-1}$) in lactose minimal agar were unsuccessful. The requirement for guanine was circumvented by constructing a prototrophic derivative of NS1063. This strain (NS1064) carried the hybrid ColE1-*gua*⁺ plasmid pLC34-10 from the Clarke & Carbon (1979) colony bank. The provision of multiple copies of a functional *gua* operon by this plasmid resulted in a basal level of IMP dehydrogenase in NS1064 5-fold greater than in the plasmid-less parental strain. Any deletions of Mu prophage from NS1064 which fuse the *lac* genes to the *gua* promoter should result in the wild-type level of expression of the fused operon. As the Mu prophage is heat-inducible, deletions were selected by plating bacteria at 42°C on lactose minimal plates. Heat-resistant Lac⁺ clones arose at a frequency of about 10^{-8} . Colonies were transferred to minimal lactose plates with and without guanine supplementation ($20 \text{ } \mu\text{g ml}^{-1}$); only those showing substantial reduction of growth in the presence of guanine were regarded as putative *gua-lac* fusions and retained for further examination.

Selection of plasmid-less strains. In order to investigate the effect of guanine starvation on the expression of the *lac* genes it was necessary to isolate derivatives of the putative *gua-lac* fusion strains that had lost pLC34-10 and were therefore guanine auxotrophs. Colonies were replicated from minimal glucose agar containing guanine to medium supplemented with xanthine. This procedure identifies bacteria that cannot convert XMP to GMP (*guaA* mutants); these arose by spontaneous loss of the plasmid at a frequency of about 2×10^{-3} . NS1065 and NS1066 are two independent *gua-lac* fusion strains from which pLC34-10 has been lost.

Characterization of *gua-lac* fusions. When the *lac* genes are fused to the *gua* regulatory region, β -galactosidase should be derepressed by guanine starvation. β -Galactosidase and IMP dehydrogenase were therefore assayed in cultures of strain NS1066 grown with guanine limitation ($4 \text{ } \mu\text{g ml}^{-1}$) or excess guanine ($40 \text{ } \mu\text{g ml}^{-1}$). IMP dehydrogenase was 48-fold derepressed by guanine depletion; this is a typical result for a *guaA* mutant (Lambden & Drabble, 1973). Excess guanine in the medium produced a 4-fold repression of the enzyme. Therefore, IMP dehydrogenase in NS1066 is controlled normally. β -Galactosidase was assayed in NS1066 growing with high and low concentrations of guanine (Fig. 1). In the

culture supplied with excess guanine ($40 \mu\text{g ml}^{-1}$) the enzyme formed a constant fraction of the total cell protein throughout the growth cycle. Guanine depletion, however, resulted in deviation from this constant value to increased production of β -galactosidase representing a 15-fold derepression of the enzyme. Subsequent addition of guanine to this derepressed culture produced an immediate repression of β -galactosidase. Derepression of β -galactosidase was reduced by 8-azaguanine (Fig. 1); this analogue is known to prevent derepression of the *gua* operon (Lambden, 1972). Hence *lacZ* appears to be controlled as an integral part of the *gua* operon in strain NS1066.

The production of β -galactosidase by the fusion strain NS1065 was also dependent on the concentration of guanine in the growth medium but, unlike NS1066, no IMP dehydrogenase activity was detectable in cell-free extracts. This indicates that the fusion in this strain results from a deletion of Mu and part or all of the *guaB* gene.

*Isolation and characterization of λ transducing phages carrying *gua-lac* fusions.* A mixed lysate was prepared from strain NS1066 by u.v.-induction and plated for single plaques in overlays of soft T-agar seeded with strain X7029 and containing X-gal. Some 30 to 40% of the plaques were blue, indicating the presence of a functional *lacZ* gene. Individual blue plaques were picked off and the phage propagated lytically on X7029. These lysates were used for transduction according to the methods of Schrenk & Weisberg (1975) with two recipients (HG1005 and PL1138) carrying *guaB* point mutations and one (KLC381) having a deletion of *guaB* (Vales *et al.*, 1979). The two point mutations lie at the extremities of the *guaB* fine-structure map, *guaB1005* (HG1005) being promoter proximal to *guaB105* (PL1138) (Gilbert & Drabble, 1980). The deletion extends from *xseA* into *guaB* and therefore removes the *gua* promoter region. The transducing phages fell into four classes. Class I transducing phages carry the whole of *guaB* and the *gua* promoter region because they transduced both point mutants and the deletion strain to prototrophy (*guaB*⁺). Classes II, III and IV do not carry the *gua* promoter (unable to transduce the deletion to *guaB*⁺). Class II phages transduced both HG1005 and PL1138, class III transduced PL1138 but not HG1005, and class IV transduced neither recipient to prototrophy. Therefore these three classes of transducing phages appear to carry different distal portions of the *guaB* gene. These results are consistent with the order promoter-*guaB*-*guaA* for the *gua* operon.

DISCUSSION

We have successfully applied the Casadaban (1976) technique to fuse *lac* genes to the *gua* operon. The major modification to the procedure was the removal of the guanine requirement of the Mu-induced *guaA* auxotroph by introducing a ColE1-*gua*⁺ plasmid prior to selection of the fusion. The modification was necessary because the *gua* operon is guanine repressible (Lambden & Drabble, 1973; Mehra & Drabble, 1981). Provision of a functional *gua* operon linked into a plasmid overcame the nutritional requirement for guanine and allowed selection of *gua-lac* fusions on minimal lactose agar. The Casadaban technique has been used to fuse *lac* genes to a variety of repressible operons of *E. coli* including *leu* (Casadaban, 1976), *purF* (Smith & Gots, 1980) and *bio* (Barker & Campbell, 1980). In none of these examples did the problem of repression of gene expression by an essential supplement in the growth medium arise.

The *gua-lac* fusions should make possible the isolation of *gua* regulatory mutants. *Gua* constitutive mutants are being sought by plating NS1066 in the presence of guanine on to lactose, melibiose (at 42 °C), or raffinose minimal media. Derepression of β -galactosidase or lactose permease is a prerequisite for growth under any of these conditions. Clones arise at a frequency of 10^{-8} ; these are being examined for constitutive expression of the *gua* and *lac* structural genes.

The λ (*gua-lac*) transducing phages are a convenient source of template DNA for studying synthesis in vitro of β -galactosidase (Zubay, 1973) under control of the *gua* promoter. λ

transducing phages of class I are known to carry the *gua* promoter and therefore provide the initiation signal for transcription. Class III phages, which lack the *gua* promoter, would serve as useful controls in these experiments. Regulation of the *gua* operon appears to be under a dual induction–repression mechanism whereby adenine nucleotides induce and guanine nucleotides repress the *gua* genes (Mehra & Drabble, 1981). The availability of a cell-free transcription–translation system now allows the testing in vitro of this hypothesis.

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