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 ColEl–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 λMu 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 μg 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 μg 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%) or soft L-agar (0.6%) agar were used.

Mutagenesis with phage Mu. Phage Mu cts62 (Howe, 1973) was spotted on to L-agar plates overlaid with soft L-agar seeded with strain X7029 [Al(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.
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(H865: thi his tyrA trp lacY gal xyl mal tonA ttx lam rpsL phx; Nijkamp & DeHaan, 1967). Recombinants (tyrA+ and his+) were selected after 30 min mating.

Isolation of λp123(209) lysogens. ip123(209) (Leathers et al., 1979) lysogens of strain X7029 with Mu inserted in gua4 were isolated by the method of Casadaban (1976) using phages λb2c and λh80cde19 (Shimada et al., 1972) and λge (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 with a frequency of about 10⁻³.

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 gua4 mutants that had acquired the pLC34-10 plasmid. The plasmid was transferred with a frequency of about 10⁻³.

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 P1clmcitr100 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 [A(lac)X741. A single plaque was resuspended in 10 ml T-broth and incubated with shaking at 37 °C for 2 h. After the addition of chloroform (1 ml), cell debris was removed by centrifugation and MgSO₄ was added to a final concentration of 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 μmol 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)X741] 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 μg ml⁻¹) of guanine in the medium resulted in derepression of IMP dehydrogenase indicating a functional guaB gene. Transduction with phage P1clmcitr100 confirmed that the mutation in NS1057 was Mu-induced. Phage P1 grown on strain W3110 (guaA₄) 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 gua₄ 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 λp123(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 λp123(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 λb2c. They were also resistant to λh80cde19, but sensitive to λgv. 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 μg ml⁻¹ (●), 4 μg ml⁻¹ (○), and 4 μg ml⁻¹ in the presence of 8-azaguanine (100 μg ml⁻¹) (△). The arrows indicate the re-addition of guanine to a final concentration of 40 μg 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 μg ml⁻¹) in lactose minimal agar were unsuccessful. The requirement for guanine was circumvented by constructing aprototrophic 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 Colonies were transferred to minimal lactose plates with and without guanine supplementation (20 μg 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. Colonies were transferred to minimal lactose plates with and without guanine supplementation (20 μg 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.

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 μg ml⁻¹) or excess guanine (40 μg 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 μg 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 ColEl-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 10⁻⁴; these are being examined for constitutive expression of the gua and lac structural genes.

The λp(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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REFERENCES


