Investigation of the Regulation of the *Escherichia coli* *btuB* Gene Using Operon Fusions

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Operon fusions were isolated between Mu dX (*lac* Cm^R Ap^R) and *btuB*, the gene encoding the multivalent vitamin B₁₂ outer membrane receptor. Using these fusions, vitamin B₁₂-mediated repression of *btuB* in *Escherichia coli* was demonstrated. Mutations in *metH*, *metE* and *ompR* as well as exogenous methionine, membrane perturbants, high osmolar conditions and temperature had no major effect on the expression of the *btuB* gene.

INTRODUCTION

In *Escherichia coli*, vitamin B₁₂ is required as a cofactor by the *metH*-encoded homocysteine transmethylese (Taylor & Weissbach, 1967) and by ethanolamine ammonia lyase (Chang & Chang, 1975). Of the two enzymes capable of methylating homocysteine to yield methionine, one is vitamin B₁₂-dependent and the second, the *metE* product, functions without the vitamin. Synthesis of the outer membrane BtuB receptor is necessary for uptake of exogenous vitamin B₁₂ (Kadner & Liggins, 1973). Previous studies have suggested that vitamin B₁₂ is involved in the repression of the *btuB* gene (Kadner, 1978), and there is good evidence that vitamin B₁₂, in the presence of the *metH* and *metF* gene products, represses the gene encoding the MetE homocysteine transmethylese (Mulligan *et al.*, 1982). In short, it would appear that under conditions where the level of vitamin B₁₂ within the bacterium is sufficient to satisfy the requirements for this cofactor (through uptake via the system involving the BtuB receptor), there is a reduction in the synthesis of both the BtuB receptor and the inefficient *metE*-encoded enzyme. In order to further our understanding of vitamin B₁₂-mediated gene regulation, we have isolated operon fusions between *btuB* and *lacZ* making use of Mu dX (*lac* Cm^R Ap^R) (Baker *et al.*, 1983). We report the effect of a number of different parameters on the control of *btuB* expression.

METHODS

The *btuB-lacZ* operon fusions were initially isolated by Mu dX (*lac* Cm^R Ap^R) infection of PAP 488 [*rpsL* Δ (*lac-pro*)] with selection for Ap^R, Cm^R and, following overnight incubation in L broth, resistance to colicin E2 on lactose–MacConkey agar plates. The Btu[–] phenotype of Lac⁺ clones was demonstrated by resistance to colicins E1 to E8 and phage BF23, and by the inability to detect the BtuB protein by SDS-PAGE of outer membrane preparations (Pugsley & Oudega, 1986). The presence of Mu dX in *btuB* was confirmed by showing that, first, P1 transduction of *btuB::Tn10* (from J.-P. Bohin, Orsay, France) resulted in replacement of the Cm^R Ap^R phenotype by the Tc^R phenotype and, second, Btu[–] and Cm^R Ap^R were cotransducible. Four independent fusions were transferred into KL320 [*metE* *his-29*(Am) *proB* *trpA605*(Am) *lacI3* *lacZ118*(Oc) *gyrA* *rpsL*; Birge & Low, 1974] by P1 phage (Silhavy *et al.*, 1984) with selection for Cm^R Ap^R and subsequent screening for the Btu[–] phenotype. That a single copy of Mu dX, inserted in *btuB*, was present was confirmed by showing that transduction of *btuB::Tn10* [source W3110 *btuB460::Tn10* from RK4936 (CGSC 6405) Δ (*argF-lac*)205 *metE70*

araD139 deoC relA1 non-9 fbbB530 rpsL btuB460::Tn10] with selection for Tc^R rendered the strains $Cm^S Ap^S$. *recA*, *metH*, *ompR* and *metE*⁺ were introduced by P1 transduction from N3072 (*recA::Tn10*; R. G. Lloyd, Nottingham, England), GW2537 [Φ (*metE-lacZ*)170 *metH174::Tn5* Δ (*lac*)U169 *rpsL*; Mulligan *et al.*, 1982], PAP1402 (*gyrA ompR885::Tn5*) and W3110 (wild-type), respectively. The F-prime F110 (*btuB*⁺) was transferred from AJ38 [*argG metB lacZ58*(Am) *recA1 rpsL* F110 *rpoB70* (Rif^R); Hayward *et al.*, 1974] by mating. Strains AJP 150, 350, 450 and 750 are the four fusion derivatives carrying *recA* and having the common genetic background KL320 *recA::Tn10 btuB::Mu dX*. AJP 151, 351, 451 and 751, respectively, represent the F110 merodiploid derivatives. Similarly, AJP 361 and 461, AJP 156, 356, 456 and 756, and AJP 158, 358, 458 and 758 are the *metH::Tn5*, *ompR::Tn5* and *metE*⁺ derivatives, respectively. The presence of *metH* was confirmed by the lack of growth on 10^{-6} M-vitamin B₁₂. The *ompR::Tn5* strains were shown to be resistant to colicin N, and to be deficient in OmpC and OmpF proteins. The latter was tested by SDS-PAGE of outer membranes prepared using the method of Pugsley & Oudega (1986). β -Galactosidase assays were done according to Miller (1972) except that the cells were lysed with 50 μ l 0.1% SDS and 100 μ l chloroform and cell debris was removed by centrifugation. The strains were grown at 37 °C in L broth or minimal M9 medium (Maniatis *et al.*, 1982) supplemented, as required, with histidine, tryptophan, proline, methionine and vitamin B₁₂. All results were obtained from duplicate assays done on three independent isolates.

RESULTS AND DISCUSSION

btuB-lacZ operon fusions were obtained *in vivo* using Mu dX (*lac* $Cm^R Ap^R$). Four independently isolated Lac⁺ fusions were transferred into KL320. $Cm^R Ap^R$ transductants were selected and screened for inability to use exogenous vitamin B₁₂, exploiting the fact that *metE* mutants grow in the absence of methionine by virtue of the vitamin B₁₂-dependent homocysteine transmethylase (*metH* product). The presence of Mu dX in *btuB* was confirmed by showing that acquisition of *btuB::Tn10* rendered the strains $Cm^S Ap^S$ (see Methods).

Exogenous vitamin B₁₂ reduced production of β -galactosidase in all *btuB-lacZ* fusion strains (Fig. 1). In general, the maximum repression ratio – the ratio of β -galactosidase activity in the presence of the highest and lowest concentration of vitamin B₁₂ – was similar for all the fusions tested. The concentration of vitamin B₁₂ capable of reducing *lacZ* expression depended upon whether a functional *btuB* gene was present. Thus, in fusions haploid for *btuB* and, consequently, lacking a functional BtuB receptor due to Mu dX insertion, the inhibitory effect of exogenous vitamin B₁₂ was only realized at concentrations in excess of 10^{-5} M. Introduction of a wild-type copy of *btuB* in *trans* on an F-prime plasmid (F110) allowed lower concentrations of exogenous vitamin B₁₂ (10^{-8} M or greater) to exert an effect (in agreement with P. D. Moir, M. G. Hunter, J. T. Armstrong and R. E. Glass, unpublished results). The maximum repression ratio was the same in both a Btu⁻ and Btu⁺ background (Fig. 1).

These experiments led us to investigate the role of a number of gene products in the regulation of *btuB*. First, because *metH* has been implicated in vitamin B₁₂-dependent regulation of *metE* (Mulligan *et al.*, 1982), we introduced a *metH::Tn5* allele into two of our fusion strains (AJP361, AJP461). Comparison of the repression ratio in a MetH⁻ background (data not shown) demonstrated that the *metH* gene did not play a major role, if any, in vitamin B₁₂-mediated regulation of *btuB*. Similar experiments were conducted for *ompR* and *metE*⁺, and similar results were obtained (data not shown); *ompR* and *metE* did not affect *btuB* expression. *ompR* was tested as it has been shown to be involved in the regulation of certain outer membrane proteins and because earlier results (Pugsley *et al.*, 1983) suggested that *ompR* mutations affected colicin E2 and vitamin B₁₂ receptor activity. The absence of any effect of *ompR* on *btuB* expression in L broth or minimal medium cultures was confirmed by demonstrating the same amounts of BtuB protein in the outer membranes of *ompR* and *ompR*⁺ cells (data not shown). In the case of *metE*, we wanted to confirm that the presence of methionine in the growth medium (necessary for the growth of *metE btuB* double mutants) did not affect *btuB* expression.

Several other potential regulatory factors of *btuB-lacZ* expression were examined. High osmolarity, local anaesthetics and *n*-alcohols are known to affect the expression of a number of genes coding for exported proteins (Pugsley, 1983). However, 300 mM-NaCl, 15 mM-phenethyl alcohol, 20 mM-procaine, 350 mM-*n*-propanol and 70 mM-*n*-butanol were without effect on steady-state *btuB-lacZ* expression in L broth cultures. Low temperature (27 °C) has also been reported to stimulate production of the BtuB protein (Lundrigan & Earhart, 1984). However, we

Fig. 1. Effect of exogenous cobalamin on β -galactosidase activity in four independently isolated *btuB-lacZ* fusions. The data is for both *Btu*⁻ [AJP150 (□), AJP350 (△), AJP450 (◇) and AJP750 (○)] and *Btu*⁺ [AJP151 (■), AJP351 (▲), AJP451 (◆) and AJP751 (●)] strains. The maximum β -galactosidase activity (in units) shown by the strains (AJP150, 101.7; AJP350, 93.9; AJP450, 90.4; AJP750, 185.6; AJP151, 98.4; AJP351, 60.2; AJP451, 46.9; AJP751, 78.3) suggests that the majority of the fusions are different. No β -galactosidase activity was found in the *recA* parent lacking a *btuB-lacZ* fusion. As a further control, we confirmed that β -galactosidase activity in a wild-type strain (W3110) was unaffected by the presence or absence of the concentration of vitamin B₁₂ employed (10⁻⁴ to 10⁻¹² M).

were unable to reproduce this low-temperature regulation of BtuB protein; incubation temperatures in the range 24–37 °C were without effect on *btuB-lacZ* expression (data not shown).

Our results confirm unambiguously that vitamin B₁₂ reduces expression of the *btuB* gene. Assuming no effect of translational control on *btuB* mRNA synthesis or stability (see Silhavy & Beckwith, 1985), the observed vitamin B₁₂-dependent alteration in β -galactosidase activity may be interpreted to reflect regulation at the transcriptional level. Approximately 75% repression was observed, a figure in close agreement with the 80–90% reduction in vitamin B₁₂ uptake capacity achieved in the presence of cobalamin (Kadner, 1978). That the concentration of exogenous vitamin B₁₂ giving this repression depended upon whether or not a functional BtuB receptor was present is in agreement with earlier work (Kadner & Liggins, 1973) which showed that *E. coli btuB* mutants are only capable of taking up vitamin B₁₂ at concentrations of 10⁻⁶ M or higher. Our results indicate that vitamin B₁₂-mediated regulation of *btuB* does not require the *metH* product. Finally, we have shown that temperature, osmolarity, membrane perturbants, methionine, and *ompR* and *metE* mutant alleles do not influence the regulation of *btuB* expression.

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