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REGULATION OF THE *cysB* GENE EXPRESSION IN *ESCHERICHIA COLI*

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It was found by Northern-type hybridization that the amount of RNA, the transcription of which begins at the *cysB* regulatory gene promoter, is significantly reduced after *cysB* gene introduction into *Escherichia coli* on multicopy plasmid. This result indicates that *cysB* protein inhibits the transcription of its own gene. *O*-acetyl-L-serine, an internal inducer of *E. coli* and *Salmonella typhimurin* cysteine regulons, has no effect on *cysB* gene expression.

The expression of regulatory genes undergoes also some kind of regulation. It is most conveniently studied through gene fusions. Autoregulation of regulatory genes is found in the great majority of cases. So far the only known exception is *malT*, the regulatory gene of maltose regulon. Its expression is positively controlled by cAMP-CRP complex. However, little is known about the level of autoregulation - whether it is transcriptional or translational. Bogasian *et al.* [1] measured the *trpR* messenger RNA level *in vitro*. They found that the presence of *trpR* gene product led to a significant decrease of the amount of *trpR* messenger.

The *cysB* gene is the regulatory gene of cysteine regulon in *Enterobacteriaceae*. The previous paper [2] reported that *cysB* gene expression is autoregulated by its own product. Recently we have found that *cysB* expression is also affected by gyrase inhibitors and urea (Bielińska & Hulanicka, in press). In this communication we present additional information about the *cysB* gene expression.

METHODS

Strains. The bacterial strains used were derived from *E. coli* K-12. They are listed in Table 1. Media and growth conditions were described previously [2].

Mutagenesis by Mu*d* (Ap′*lac*) phage was performed as described by Casadaban

Table 1

List of strains

Strain	Genotype	Source
MC4100	*araD*139 *Δ(lac)U*169 *strA relA thi*	M. J. Casadaban *via* M. Iaccarino
EC1250	*araD*139 *Δ(lac)U*169 *strA relA thi trp*	Jagura-Burdzy & Hulanicka 1981
EC1173	*araD*139 *Δ(lac)U*169 *strA relA thi trp cysB88*::Mud(Ap'*lac*)	This work
EC1848	EC1173 *cysE* Tn10	„
EC1855	EC1173/pASH2	„
EC1856	EC1848/pASH2	„
EC1851	EC1173/KLF123	„
EC1852	EC1848/KLF123	„
EC1163	*leu6ΔtrpE5 thi cysE* Tn10 $r^- m^+$	„
CGSC4256	*pyrD*34 *trp*45 *his*68 *recA*1 *galK*35 *xyl*-7 *mtl*-2 *malA*1 *rpsL*118/KLF123 $pyrF^+$ $cysB^+$ trp^+	*E. coli* Genetic Stock Center

& Cohen [3]. Transformation was carried out according to Kushner [4]. F'-Mediated conjugation and transduction were performed according to Miller [5].

Isolation of RNA and plasmid DNA. RNA was isolated as described by Ikemura & Dahlberg [6]. Plasmid DNA was isolated by the method of Holmes & Quigley [7].

Plasmid plG400 DNA was labelled by "nick translation" [8]. Hybridization was done by the method of Wahl *et al.* [9].

β-Galactosidase assay. The enzymatic activity of β-galactosidase was assayed in toluenized cells by the method of Miller [5].

Construction of the double mutant cysE cysB:: *lac*. The insertion of the Mu phage into *cysB* gene results in cysteine auxotrophy. Therefore it was impossible to achieve direct integration of the second cys^- mutation. To overcome this obstacle we prepared the lysate of P1 phage on the strain which carried Tn10 transposon linked to *cysE* mutation. The *cysB*:: *lac* strain was transducted with this phage lysate to tetracycline resistance. Some of the tetracycline resistant transductants were double mutants; these colonies would not give cysteine prototrophs in cross with WT phage lysate, since double transductions in one cross are unlikely. To eliminate the colonies with only one cysteine mutation (*cysB*:: *lac*) the transduction with P1 lysate on WT strain was performed. Among transductants which did not give cys^+ colonies in this cross were those carrying *cysE cysB*:: *lac* mutations. The presence of *cysE* mutation was confirmed by the assay of serine transacetylase the enzyme coded for by *cysE* gene. The introduction of *cysE* mutation into the strain *cysB*:: *lac* carrying the *cysB* gene on the plasmid pASH2 (cysteine prototroph) was performed directly by P1 transduction to tetracycline resistance and cysteine auxotrophy.

RESULTS AND DISCUSSION

The effect of the cysB gene on its own transcription. It was of interest to check whether the presence of *cysB*$^+$ allele on the plasmid affects the amount of messenger RNA directed by the promoter region of the *cysB* gene. For this purpose the total RNA from the fusion strains *cysB88*:: *lac* (EC1173) and *cysB88*:: *lac*/pASH2 (EC1855) was isolated, separated on the agarose formaldehyde gel, then transferred to nitrocellulose paper. Plasmid pASH2 carries the *cysB* gene of *S. typhimurium* cloned in the pBR325 vector. Nick translated DNA of plasmid plG400 which contained the *lac* operon without promoter region, was used as a probe for identifi-

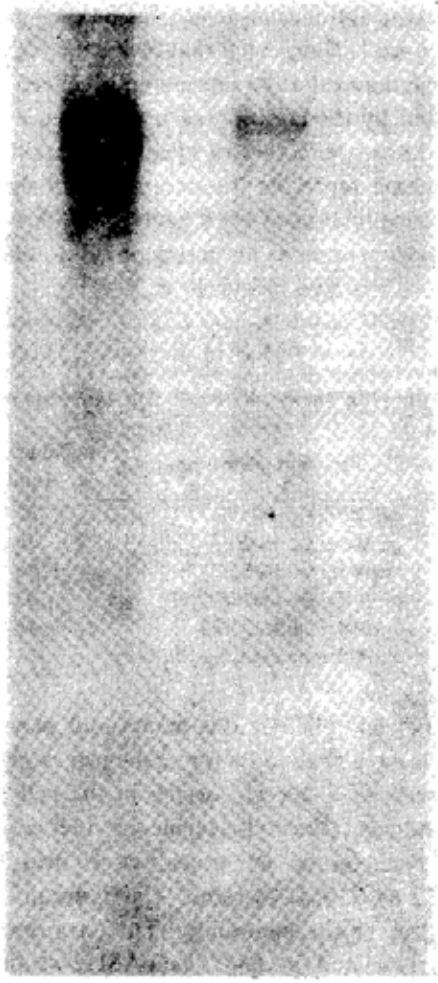

Plate 1. Hybridization of RNA isolated from strains EC1173 and EC1855 to DNA of plasmid plG400. Lane 1, RNA isolated from *cysB88: lac* (EC1173); lane 2, RNA isolated from *cysB88: : lac*/pASH2 (EC1855).

cation of the *lac* mRNA transcript initiated in the regulatory region of the *cysB* gene [10]. The amount of mRNA visualized by autoradiography was significantly lower in the fusion strain carrying the *cysB*$^+$ plasmid, pASH2 (Plate I). This result indicates that the *cysB* protein repressed its own synthesis on the level of transcription. Of course these experiments do not exclude a possibility of another type of control, for example: inefficient translation of mRNA, as it was reported for *malT* gene expression [11].

Studies on the effect of the internal inducer (*O*-acetyl-L-serine) on the *cysB* gene expression. Recently it was reported that the regulation of the expression of some regulatory genes depends on the low molecular effectors. Aiba [12] showed that CRP protein is a transcriptional repressor for its own gene only in the presence of cAMP. McFall & Heinicz [13] reported that *dsdC*, the regulatory gene of D-serine deaminase, is autoregulated and that its repression is relived by D-serine. Repression of *trpR* gene is stimulated by the addition of tryptophan, a corepressor of *trp* operon [1]. There are two known effectors of cysteine regulon; L-cysteine and *O*-acetyl-L-serine [14]. L'-Cysteine represses the expression of cysteine ctructural genes, but has no effect on the expression of *cysB* gene [2]. *O*-Acetyl-L-serine is necessary for functioning of the *cysB* protein as an activator of cysteine regulon, being therefore an internal inducer of cysteine biosynthesis.

Table 2

Effect of cysE mutation on the expression and autoregulation of cysB gene

Strain	Pertinent genotype	β-Galactosidase (units)
EC1173	*cysB88:: lac*	732
EC1848	*cysB88:: lac cysE*	716
EC1855	*cysB88:: lac*/pASH2	98
EC1856	*cysB88:: lac cysE*/pASH2	93
EC1851	*cysB88:: lac*/KLF123	96
EC1852	*cysB88:: lac cysE*/KLF123	101

It was of interest to check whether this compound plays also some role in the *cysB* gene expression. The *cysE* gene codes for serine transacetylase, the enzyme which is responsible for synthesis of *O*-acetyl-L-serine in the cell [14]. In order to study the effect of internal inducer *O*-acetyl-L-serine on the *cysB* gene expression, the *cysE* mutation was introduced by P1 transduction into *cysB88:: lac* (EC1173) and *cysB88:: lac*/pASH2 (EC1855) strains. *CysE* mutation was introduced by multiplying phage on the strain containing Tn10 transposon linked with *cysE* (EC1163). The *cysB88:: lac* and *cysB88:: lac*/pASH2 strains were transduced to tetracycline resistance and among transductants *cysE* mutation was scored (see Materials and Methods). The effect of *cysE* mutation on the *cysB* gene expression and its repression by its own protein was checked by the assays of β-galactosidase

in fusion strains (Table 2). The level of β-galactosidase in all studied strains was not affected by the presence of the *cysE* mutation. The *cysB* gene on plasmid pASH2 derives from *S. typhimurium* whereas the *cysB88*:: *lac* is in *E. coli* strain. It is known that the *cysB* protein of *S. typhimurium* acts as an activator in *E. coli* strains and *vice versa* [2]. To check whether the lack of the effect of *O*-acetyl-L-serine on *cysB* gene expression could be due to *cysB* protein being derived from *S. typhimurium*, episome KLF123 was introduced by F'-mediated conjugation and β-galactosidase was assayed in the parential and merodiploid strains (Table 2). Similarly as in transformants, the level of β-galactosidase was found to be the same. Therefore *O*-acetyl-L-serine is dispensable both for functioning of the *cysB* protein as an autorepressor and the *cysB* gene expression.

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