Silencing of the *Escherichia coli* *bgl* operon by RpoS requires Crl

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Silencing of the *Escherichia coli* *bgl* operon is mediated by histone-like protein H-NS and affected by other pleiotropic regulators, including sigma factor RpoS. Silencing is relieved and the *bgl* operon is activated in *hns* mutants and by mutations that map in the vicinity of the *bgl* promoter. However, the expression level of activated *bgl* operon derivatives varies with the strain background. Here it is shown that the repression of the *bgl* operon by RpoS requires Crl. Crl is a protein that is necessary for the RpoS-dependent expression of the *csgBA* operon and that enhances the expression of other RpoS-dependent genes. In a Crl-negative strain RpoS had no effect on the *bgl* operon. The *crl* gene maps close to the *proBA* locus in the *lac* operon region and is deleted in many commonly used *E. coli* strains. Crl may therefore account for some of the observed strain-dependent variations of *bgl* operon expression levels and effects of pleiotropic regulators on *bgl* operon regulation.

Keywords: β -glucoside, pleiotropic regulation, H-NS, *lac* operon region

INTRODUCTION

The *bgl* operon, encoding the gene products necessary for the fermentation of aryl- β -glucosides, is silent in wild-type *Escherichia coli* (Schaeffler & Maas, 1967). The abundant histone-like nucleoid-structuring protein H-NS is essential for *bgl* silencing and in *hns*-null strains the operon is expressed at high levels (Defez & de Felice, 1981; Higgins *et al.*, 1988). The activity of the CRP/cAMP-dependent *bgl* promoter is also enhanced and expression of the operon is activated by spontaneous mutations that disrupt a silencer sequence located upstream of the promoter, and by point mutations that improve the CRP-binding site (Reynolds *et al.*, 1981, 1986; Schnetz & Rak, 1992; Singh *et al.*, 1995). Silencer sequences located downstream of the promoter contribute to silencing of the operon (Schnetz, 1995).

The expression level of the activated *bgl* operon derivatives and the effect of mutations that partially disrupt the *hns* gene can vary with the strain background (Higgins *et al.*, 1988; Lopilato & Wright, 1990; Tsui *et al.*, 1994; Free *et al.*, 2001). In addition to H-NS and CRP, the *bgl* operon is affected by other pleiotropic regulators including the DNA-bending protein FIS, which competes with CRP for binding (Caramel & Schnetz, 2000), and the transcriptional-regulator-like proteins LeuO and BglJ (Giel *et al.*, 1996; Ueguchi *et al.*, 1998). RpoS, the key regulator in the stress response of *E. coli* and the H-NS homologue StpA are necessary for

silencing of the wild-type *bgl* operon by a truncated H-NS protein lacking its C-terminal DNA-binding domain (Free *et al.*, 1998, 2001; Ohta *et al.*, 1999). Furthermore, RpoS downregulates the expression of activated *bgl* operon alleles (Dole *et al.*, 2002). Interestingly, the up to 50-fold repression of the *bgl* operon by RpoS is based on the amplification of a moderate (two- to threefold) repression of the transcription rate by RpoS via a second, post-transcriptional level of regulation involving the specific antiterminator protein BglG. BglG is encoded by the first gene of the operon. At low transcription rates BglG is limiting and transcription halts at rho-independent transcriptional terminators *t1* in the leader and *t2* within the operon. If the transcription rate increases above a threshold the basal synthesis of BglG is sufficient for antitermination and, as a result, *bglG* and the operon are expressed at high levels (Dole *et al.*, 2002).

RpoS activity is controlled by multiple signals and at various levels. Predominant is the post-transcriptional control, for example its translation is regulated by the RNA-binding protein Hfq and small regulatory RNAs and its proteolysis is controlled by the response regulator RssB and the ClpXP protease (Hengge-Aronis, 2000). In addition, RpoS activity is affected by Crl, a 15 kDa protein that is conserved among the *Enterobacteriaceae* (for example, it is present in the complete genome sequences of *Salmonella enterica*, *Salmonella typhimurium* LT2, *Yersinia pestis* and *Vibrio cholerae*) but

Table 1. *E. coli* K-12 strains

The relevant genotype of the constructed CSH50 derivatives refers to the *bgl* and *rpoS* loci, and the *gpt-lac* region. Mutations causing activation of the silent *bgl* operon include *bgl*-CRP (a C to T exchange in the CRP binding site at position –66 relative to the transcription start), *bgl*::IS1-R1243 (integration of IS1 in orientation II generating a target site duplication from –88 to –80), *bgl*::IS5-H3 (an integration of IS5 in orientation II generating a target site duplication from position –92 to –89) and *bgl*-Δ2 (a deletion of the upstream silencer, extending from position –77). Terminator mutation *t1-L* carries a three base exchange in the left stem of the inverted repeat structure (Dole *et al.*, 2002). *bgl*⁰ indicates a silent wild type *bgl* operon.

Strain	Relevant genotype or structure	Construction*/reference
CSH50	<i>bgl</i> ⁰ Δ(<i>lac-pro</i>) <i>ara thi</i> (=S49)	Miller (1972)
R1243	CSH50 <i>bgl</i> ::IS1-R1243 (Bgl ⁺) (=S157)	Schnetz & Rak (1992)
RH90	MC4100 <i>bgl</i> ⁰ <i>rpoS</i> 359::Tn10 (=S810)	Lange & Hengge-Aronis (1991)
S162	CSH50 Δ <i>bgl</i> -AC11	Caramel & Schnetz (1998)
S308	CSH50 <i>attB</i> ::[SpecR <i>lacOP lacZ</i>]	× pKESD13
S432	CSH50 <i>bgl</i> -CRP	Spontaneous Bgl ⁺ mutant
S484	CSH50 <i>bgl</i> ⁰ Δ(<i>argF-lac</i>)U169 Pro ⁺	Dole <i>et al.</i> (2002)
S524	CSH50 <i>bgl</i> ⁰ Δ <i>lacZ</i> -Y217 [<i>gpt-lac</i>] ⁺	Dole <i>et al.</i> (2002)
S541	S524 Δ <i>bgl</i> -AC11	Dole <i>et al.</i> (2002)
S544	S524 <i>bgl</i> -CRP	Dole <i>et al.</i> (2002)
S887	S541- <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1071	S524 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1079	S544 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1142	S541 <i>attB</i> ::[SpecR WT P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK11; Dole <i>et al.</i> (2002)
S1144	S541 <i>attB</i> ::[SpecR <i>bgl</i> -CRP P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK12; Dole <i>et al.</i> (2002)
S1146	S541 <i>attB</i> ::[SpecR <i>bgl</i> -Δ2 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK13; Dole <i>et al.</i> (2002)
S1148	S541 <i>attB</i> ::[SpecR <i>bgl</i> ::IS5-H3 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK14; Dole <i>et al.</i> (2002)
S1155	S1142 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1157	S1144 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1159	S1146 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1161	S1148 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1163	S162 <i>attB</i> ::[SpecR WT P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK11
S1165	S162 <i>attB</i> ::[SpecR <i>bgl</i> -CRP P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK12
S1167	S162 <i>attB</i> ::[SpecR <i>bgl</i> -Δ2 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK13
S1169	S162 <i>attB</i> ::[SpecR <i>bgl</i> ::IS5-H3 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK14
S1171	S1163 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1173	S1165 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1177	S1169 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1245	S524 <i>bgl</i> ::IS1-R1243 [Bgl ⁺]	Dole <i>et al.</i> (2002)
S1250	S1245 <i>rpoS</i> 359::Tn10	Dole <i>et al.</i> (2002)
S1274	S541 <i>attB</i> ::[SpecR <i>lacOP lacZ</i>]	× pKESD13
S1276	S887 <i>attB</i> ::[SpecR <i>lacOP lacZ</i>]	× pKESD13
S1305	S308 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1484	CSH50 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1486	S162 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1489	S484 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1491	S432 Δ(<i>argF-lac</i>)U169 Pro ⁺	× T4GT7(MC4100)
S1493	R1243 Δ(<i>argF-lac</i>)U169 Pro ⁺	× T4GT7(MC4100)
S1495	R1243 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1497	S432 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1499	S1491 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1501	S1493 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)
S1503	S541 <i>attB</i> ::[SpecR <i>bgl</i> ::IS1-R1243 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK20
S1505	S162 <i>attB</i> ::[SpecR <i>bgl</i> ::IS1-R1243 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK20
S1507	S887 <i>attB</i> ::[SpecR <i>bgl</i> ::IS1-R1243 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK20
S1508	S1486 <i>attB</i> ::[SpecR <i>bgl</i> ::IS1-R1243 P _{<i>bgl</i>} <i>t1-L bglG lacZ</i>]	× pKESK20
S1512	S1167 <i>rpoS</i> 359::Tn10	× T4GT7(RH90)

* × indicates that transductions and integrations into *attB* were performed as described in Methods.

shares no homology with other proteins. Crl was discovered because it is required for the RpoS-dependent expression of the *csgBA* operon encoding the fibronectin-binding curli fimbria (Crl = curli) (Olsen *et al.*, 1989; Arnquist *et al.*, 1992). Genetic analyses revealed that Crl stimulates to various extents all RpoS-dependent transcription processes and that Crl acts either in concert with RpoS or upstream of it in the same pathway (Pratt & Silhavy, 1998).

Here the expression level of activated *bgl* operon derivatives and *bgl-lacZ* reporter constructs were tested in strain CSH50 which carries a large deletion in the *lac* region, $\Delta(gpt-lac)$, and derivatives that carry the smaller $\Delta(argF-lac)$ deletion or the wild-type *gpt-pro-lac* ($\Delta lacZ$) region. In strain CSH50, activated *bgl* operon alleles are expressed at high levels. However, their expression is low in the other two deletion derivatives. In these strains the expression is increased up to 50-fold when a *rpoS* mutation is introduced (see also Dole *et al.*, 2002). RpoS has no effect in CSH50. A complementation experiment shows that Crl, which is encoded in the *gpt-argF* region, is required for the RpoS-dependent repression of the *bgl* operon and responsible for these strain-dependent variations in the expression level of the *bgl* operon.

METHODS

Strains and plasmids. Plates and liquid media were used as described by Dole *et al.* (2002). The genotypes of the *E. coli* K-12 strains used in this study are listed in Table 1. All strains are derivatives of CSH50 (Miller, 1972). [The $\Delta(pro-lac)$ X111 deletion present in CSH strains was mapped and renamed as $\Delta(gpt-lac)$, Genetic Stock Center, <http://cgsc.biology.yale.edu/>] Transductions were performed with phage T4GT7 (Wilson *et al.*, 1979). For transduction of *rpoS359::Tn10*, cells were selected on LB tetracycline ($12 \mu\text{g ml}^{-1}$) plates and for transduction of $\Delta(argF-lac)$ (Pro⁺) cells, minimal glucose B1 plates were used. Integrations of *bgl-lacZ* reporter constructs into the chromosomal phage lambda attachment site *attB* using plasmids pKESK11, pKESK12, pKESK13, pKESK14, pKESK20 or pKESD13 were performed and confirmed by PCR as described (Diederich *et al.*, 1992; Dole *et al.*, 2002). Plasmids were constructed according to standard techniques (Sambrook *et al.*, 1989). The structure of plasmids pKESD13 and pKESD20 is similar to pKESK11 (Dole *et al.*, 2002). Plasmid pKESD13 carries the *lac* operator promoter and the *lacZ* gene cloned into a pACYC backbone, including the kanamycin-resistance *neo* gene, *attP*, and the spectinomycin-resistance *aadA* gene. Plasmid pKESK20 carries the *bgl* promoter activated by integration of IS1 (allele *bgl-R1243*) (Schnetzer & Rak, 1992), *bgl* terminator mutant *t1-L*, *bglG* and *lacZ*. Plasmid pKESK19 carries the *crl* gene cloned under control of the constitutive antisense promoter of the tetracycline resistance gene (Stüber & Bujard, 1981). For its construction the *crl* gene fragment was amplified by PCR and cloned into pBR322, replacing the *bla* gene. In addition, a kanamycin resistance gene cassette was inserted into the *tet* gene. PCR fragments were sequenced after cloning. Details of constructions and compiled sequences of the plasmids are available upon request.

Phospho- β -glucosidase and β -galactosidase assays. These were performed as described by Miller (1972), Schnetz & Rak (1988) and Dole *et al.* (2002). Cells were grown in M9 medium

containing 1% glycerol and 0.66% Casamino acids (Difco). For induction of the *bgl* operon, 0.5% (w/v) salicin was added in the phospho- β -glucosidase assays. Cultures were inoculated from non-induced fresh overnight cultures grown in the same medium and cells were harvested after approximately 3 h growth at 37 °C at OD₆₀₀ of 0.5. Enzyme activities were determined at least three times from at least two independent transformants or integration derivatives. Standard deviations were less than 10% in case of the β -galactosidase assays and less than 20% in case of the phospho- β -glucosidase assays.

RESULTS AND DISCUSSION

Expression levels of activated *bgl* operon derivatives are affected by deletions in the *lac* operon region

The expression levels of the wild-type and activated *bgl* operon alleles were tested in a set of isogenic CSH50 derivatives including a $\Delta(gpt-lac)$, a $\Delta(argF-lac)$ and a $\Delta lacZ$ deletion (Fig. 1). The activated alleles of the *bgl* operon that were tested include a mutant carrying an improved CRP-binding site or an integration of IS1 (Fig. 2). The expression level of the *bgl* operon was determined in phospho- β -glucosidase assays from exponential-phase cultures grown in minimal M9 glycerol medium containing salicin as inducer. Phospho- β -glucosidase is encoded by the third gene of the *bgl* operon (*bglB*).

As expected, the expression level of the silent wild-type *bgl* operon was low in all strains tested (Fig. 2a). However, the expression level of activated alleles varied significantly with the CSH50 strain and its deletion derivatives. These alleles are expressed at low levels in the $\Delta lacZ$ and $\Delta(argF-lac)$ strains (Fig. 2). However, when the isogenic $\Delta(gpt-lac)$ strain was used, the activated alleles were expressed at high levels. Expression of the IS1-activated allele is higher than that of the allele carrying an improved CRP-binding site. Low expression of activated alleles in the $\Delta lacZ$ strain when cells are grown in medium containing glycerol was observed previously (Dole *et al.*, 2002). Glycerol causes some catabolite repression of the operon (Lopilato & Wright, 1990). Irrespective of the background, all strains carrying an activated operon show a Bgl-positive phenotype on salicin indicator plates. In the $\Delta lacZ$ and $\Delta(argF-lac)$ strain backgrounds, the introduction of a mutation in *rpoS* caused an up to 50-fold increase in the expression of the activated alleles (Fig. 2) (see also Dole *et al.*, 2002), while RpoS had no further effect in the $\Delta(gpt-lac)$ strain (Fig. 2). This indicated that a gene in the *gpt-argF* region is required for repression of the *bgl* operon by RpoS (Fig. 1).

Crl is required for the RpoS-mediated repression of the *bgl* operon

The *crl* gene maps in the *gpt-argF* region and thus was an obvious candidate for the observed difference. To test whether Crl complements the $\Delta(gpt-lac)$ strain, plasmid pKESK19 carrying the *crl* gene under control of the constitutive antisense promoter of the tetracycline resistance gene (Stüber & Bujard, 1981) was constructed.

Fig. 1. Chromosomal map of the *gpt-pro-lac* operon region. Expression of the *bgl* operon was tested in strain background CSH50 (Miller, 1972) carrying a large deletion in the *lac* operon region $\Delta(gpt-lac)$, and CSH50-derived strains that carry a smaller deletion in the *lac* region, $\Delta(argF-lac)$, or a deletion of the *lacZ* gene only (Dole *et al.*, 2002). Map positions are given in kbp (*gpt* is at 256 kbp, *crl* is at 257.8 kbp, *proBA* is at 260.0 kbp, *argF* is at 289.5 and *lac* is at 365.5 kbp).

	β -Glucosidase activity							
	$\Delta lacZ$		$\Delta(argF-lac)$		$\Delta(gpt-crl-lac)$			
	-	<i>rpoS</i>	-	<i>rpoS</i>	-	<i>rpoS</i>	-	<i>rpoS</i>
(a) sil CRP ^P t1 t2 <i>bglG</i> <i>bglF</i> <i>bglB</i>	1	1	2	2	2	2	2	2
(b) CRP ⁺	2	80	2	25	40	30	3	16
(c) IS1	2	100	5	93	135	100	6	55

Fig. 2. RpoS and Crl are mutually required for repression of activated *bgl* operon alleles. Expression levels of (a) the wild-type *bgl* operon and alleles activated by (b) improvement of the CRP-binding site (CRP⁺, a C to T exchange at position -66 relative to the transcription start site), and (c) integration of IS1 (*bgl*::IS1-R1243) (Dole *et al.*, 2002) are given in units of phospho- β -glucosidase activity. Phospho- β -glucosidase is encoded by *BglB*. *BglF* encodes the β -glucoside specific permease enzyme II^{Bgl}. Stem-loop structures *t1* and *t2* are rho-independent terminators at which *BglG* mediates antitermination. Expression levels were determined in CSH50 and derivatives, which differ in the size of the deletion in the *lac* operon region [$\Delta(gpt-lac)$, $\Delta(argF-lac)$ and $\Delta lacZ$]. Also tested were *rpoS359::Tn10* (Lange & Hengge-Aronis, 1991) mutants of CSH50 and its derivatives (Table 1). +Crl indicates expression levels of transformants of the *crl* expressing plasmid pKESK19. Strains used were: (a) S524, S1071, S484, S1489, CSH50 and S1484; (b) S544, S1079, S1491, S1499, S432 and S1497; (c) S1245, S1250, S1493, S1501, R1243 and S1495.

In transformants of the $\Delta(gpt-lac)$ strain the repression of the activated *bgl* operon alleles by RpoS was restored (Fig. 2; +Crl). As a control, in the RpoS-negative $\Delta(gpt-lac)$ strain providing Crl *in trans* caused a less than twofold decrease [Fig. 2; $\Delta(gpt-crl-lac)$ *rpoS* + Crl]. This demonstrates that Crl is required for the RpoS-mediated repression.

Crl is required for the RpoS-mediated repression of *bgl-lacZ* reporter constructs

Next a chromosomal *bgl-lacZ* reporter system was used in which expression is independent of the β -glucoside specific regulation by *BglG*-mediated antitermination due to a mutation (*t1-L*) of terminator *t1* (Fig. 3) (Dole *et al.*, 2002). In this system the changes in the transcription rate are not amplified by *BglG*-mediated antitermination and thus correlate with changes in the levels of expressed β -galactosidase (see Introduction). RpoS has an approximately threefold effect on the expression level of the *bgl-lacZ* reporter gene system

	β -Galactosidase activity							
	$\Delta lacZ$		$\Delta(gpt-crl-lac)$					
	-	<i>rpoS</i>	-	<i>rpoS</i>	-	<i>rpoS</i>	-	<i>rpoS</i>
(a) sil CRP ^P t1-L <i>bglG</i> <i>lacZ</i>	12	27	25	24	10	24		
(b) CRP ⁺	265	660	708	705	160	675		
(c) IS1	1000	1980	1730	2180	575	2070		
(d) IS5	370	1050	1120	1327	243	1126		
(e) Δ	150	470	460	463	85	425		
(f) Plac _{attB} <i>lacZ</i>	5650	6450	8000	9850	4950	9430		

Fig. 3. Crl is required for the RpoS-mediated repression of *BglG*-independent *bgl-lacZ* reporter constructs. Chromosomally encoded *bgl-lacZ* reporter constructs (Dole *et al.*, 2002) carrying the wild-type *bgl* promoter (a) or alleles activated by (b) improvement of the CRP-binding site (CRP⁺), (c) integration of IS1, (d) integration of IS5 and (e) a deletion of the upstream silencer (Δ 2 in Table 1) were tested in the CSH50 $\Delta lacZ$ derivative and in CSH50, $\Delta(gpt-crl-lac)$ [*rpoS*⁺], and the *rpoS359::Tn10* mutant derivatives of these strains. Crl was provided *in trans* (+Crl) using plasmid pKESK19. All the *bgl-lacZ* constructs carry mutation *t1-L* of the first terminator, a 3 bp exchange in the left stem of the inverted repeat forming the terminator, which renders expression independent of *BglG*-mediated antitermination. As a control (f) expression of a *lacOP-lacZ* construct integrated into *attB* (Diederich *et al.*, 1992; Dole *et al.*, 2002) was also tested. Strains used were: (a) S1142 ($\Delta lacZ$), S1155 ($\Delta lacZ$ *rpoS359::Tn10*), S1163 ($\Delta gpt-lac$), and S1171 ($\Delta(gpt-lac)$ *rpoS359::Tn10*); (b) S1144, S1157, S1165 and S1173; (c) S1503, S1507, S1505 and S1508; (d) S1146, S1159, S1167 and S1512; and (e) S1274, S1276, S308 and S1305.

(Dole *et al.*, 2002). Analysed were *bgl-lacZ* fusions that carry the wild-type *bgl* promoter (Fig. 3a) or activated alleles, including the mutants that carry the improved CRP-binding site (Fig. 3b) or the integration of IS1 (Fig. 3c). In addition, mutants carrying an integration of IS5 (Fig. 3d) and a deletion of the upstream silencer were tested (Fig. 3e) (Dole *et al.*, 2002). Expression levels are given in units of β -galactosidase activity and thus the values are higher than in the phospho- β -glucosidase assay shown in Fig. 2. Similar to the results obtained using the *bgl* operon, the *bgl-lacZ* constructs were repressed by RpoS when Crl was present ($\Delta lacZ$) but not when Crl was missing ($\Delta(gpt-crl-lac)$) (Fig. 3). Repression

of the activated alleles by RpoS is approximately two- to threefold (Fig. 3). Again, providing Crl *in trans* restored repression by RpoS in the $\Delta gpt\text{-}crl\text{-}lac$ strain, but had no effect in the *rpoS* mutant of this strain (Fig. 3). As a control, the expression of a *lacOP-lacZ* fragment integrated into *attB* varied only moderately in the various strain backgrounds (Fig. 3).

Role of RpoS and Crl in *bgl* operon regulation

The *bgl* operon is regulated by various pleiotropic regulators including H-NS and CRP, as essential factors. In addition, rather moderate changes (approximately two- to threefold) in the transcription rate of the operon can be amplified post-transcriptionally into large changes in the expression level (Dole *et al.*, 2002). Therefore, the expression level of the *bgl* operon and the effect of any non-essential factor (for example RpoS and Crl) involved in *bgl* operon regulation may vary significantly depending on the strain background and the growth conditions that are used. RpoS causes a moderate repression of the *bgl* promoter of activated *bgl* operon derivatives (Dole *et al.*, 2002). The finding that the RpoS-mediated repression requires Crl supports earlier findings that Crl stimulates RpoS activity. How Crl stimulates RpoS is unknown. Interestingly, RpoS is required for silencing of a *bgl-lacZ* fusion by a truncated H-NS protein in a $\Delta(gpt\text{-}lac)$, i.e. Crl-negative, strain (Ohta *et al.*, 1999). Likewise, the contribution of Crl to the RpoS-mediated transcription of the Curli (*csgBA*) operon varies with the strain background (Olsen *et al.*, 1989; Provence & Curtiss, 1992). This indicates that the enhancement of RpoS-mediated regulation by Crl may depend on H-NS and other pleiotropic regulators.

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