Independent regulation of H-NS-mediated silencing of the *bgl* operon at two levels: upstream by BglJ and LeuO and downstream by DnaKJ

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Silencing of the *Escherichia coli bgl* operon by the histone-like nucleoid-structuring protein H-NS occurs at two levels. Binding of H-NS upstream of the promoter represses transcription initiation, whilst binding within the coding region is also proposed to repress transcription elongation. The latter, downstream level of repression is counteracted by the protease Lon and, thus, silencing of the *bgl* operon is more effective in *lon* mutants. Transposon-mutagenesis screens for suppression of this *lon* phenotype on *bgl* were performed and insertion mutations disrupting *rpoS* and *crl* were obtained, as well as mutations mapping upstream of the open reading frames of *bglJ*, *leuO* and *dnaK*. In *rpoS* and *crl* mutants, *bgl* promoter activity is known to be higher. Likewise, as shown here, *bgl* promoter activity is increased in the *bglJ* and *leuO* mutants, which express BglJ and LeuO constitutively. However, BglJ and LeuO have no impact on downstream repression. A *dnaKJ* mutant was isolated for the first time in the context of the *bgl* operon. The mutant expresses lower levels of DnaK than the wild-type. Interestingly, in this *dnaKJ::miniTn10* mutant, downstream repression of *bgl* by H-NS is less effective, whilst upstream repression by H-NS remains unaffected. Together, the data show that the two levels of *bgl* silencing by H-NS are regulated independently.

Received 31 March 2005

Revised 20 July 2005

Accepted 21 July 2005

INTRODUCTION

The abundant histone-like nucleoid-structuring protein H-NS is a key regulator in the adaptation of *Escherichia coli* to its environment (Usery *et al.*, 1994; Schröder & Wagner, 2002) and it affects the expression of ~5% of the genes in *E. coli* K-12 (Hommals *et al.*, 2001). The current view of the mechanism of H-NS-mediated repression is that H-NS binds as a dimer to AT-rich and planar-bent DNA sequences and then forms an extended oligomeric complex on the DNA, which represses transcription initiation when located next to a promoter. Efficient repression by H-NS often involves binding of H-NS to two close binding (or nucleation) sites and probably the formation of a DNA loop, by 'zipping' the two sites together (Dame *et al.*, 2002; Dorman, 2004). In several H-NS-repressed systems analysed so far, the H-NS-mediated repression is relieved by specific mechanisms that disturb the formation of the repressing complex, for example by binding of a specific transcription factor or by a temperature-dependent change in the DNA bend (Dorman, 2004; Prosseda *et al.*, 2004). To date, little is known about whether and how the activity of the H-NS protein itself and its capacity to form repressing complexes are modulated. One possibility is that the formation of heterodimers and hetero-oligomers between H-NS and its

homologue StpA affects its activity (Williams *et al.*, 1996; Johansson & Uhlin, 1999; Johansson *et al.*, 2001). Interaction of H-NS with Hha and Hfq has also been reported (Kajitani & Ishihama, 1991; Nieto *et al.*, 2000). Furthermore, it was found that *hscA*, encoding an HSP66 protein and DnaK homologue, modulates the H-NS-mediated repression of *pilA* and the *bgl* operon in an *hns* mutant with reduced levels of H-NS protein (Kawula & Lelivelt, 1994). H-NS may also be modulated by a post-translational modification with short-chain poly(R)-3-hydroxybutyrate (Reusch *et al.*, 2002).

The *E. coli bgl* operon is one example of a system whose repression by H-NS is exceptionally specific. The *bgl* operon encodes the gene products for the fermentation of aryl β -D-glucosides, including a β -glucoside-specific permease EII^{Bgl} (or BglF) and a phospho- β -D-glucosidase BglB (Fig. 1b). Also encoded within the operon is the positive regulator and anti-terminator protein BglG, whose activity is regulated by phosphorylation and which, under inducing conditions, allows transcription elongation beyond two terminators (t1 and t2) within the operon (Amster-Choder & Wright, 1993; Görke, 2003).

Silencing of the *bgl* operon by H-NS occurs under all laboratory growth conditions tested (Prasad & Schaefer, 1974; Reynolds *et al.*, 1981; Lopilato & Wright, 1990).

Abbreviation: H-NS, histone-like nucleoid-structuring protein.

Silencing involves two sites (Dole *et al.*, 2004b): H-NS represses transcription initiation at the cAMP receptor protein (CRP)-dependent promoter by binding to an AT-rich and presumably bent upstream silencer sequence (Schnetz, 1995; Singh *et al.*, 1995; Schnetz & Wang, 1996; Mukerji & Mahadevan, 1997). In addition, H-NS binds to a site located 600–700 bp downstream of the transcription-initiation site within the coding region of the first gene, *bglG*. Binding to this downstream silencer is likely to hinder transcription elongation (Schnetz, 1995; Dole *et al.*, 2004b). Silencing of *bgl* can be relieved by mutations that disrupt the upstream H-NS-binding site or that improve the CRP-binding site (Reynolds *et al.*, 1986; Schnetz & Rak, 1988; Singh *et al.*, 1995). Recently, we found that the silencing by H-NS mediated via the downstream site ('downstream repression') is more efficient in *lon* mutants (Dole *et al.*, 2004a), suggesting that it is modulated by the ATP-dependent Lon protease (Gottesman, 1996). Therefore, a *bgl* allele (*bgl*-CRP⁺) that escapes silencing in the wild-type due to a mutation that improves the CRP binding of the *bgl* promoter remains repressed in a *lon* mutant (Dole *et al.*, 2004a).

In this work, we screened for suppressors of the more effective downstream repression of *bgl* by H-NS in a *lon*

Fig. 1. Mutagenesis screen for suppressors of efficient *bgl* operon silencing in *lon* mutants. (a) Plasmid pKESK18 was used to perform a transposon-mutagenesis screen to isolate suppressors of the more effective repression of *bgl* in *lon* mutants. Plasmid pKESK18 is a replication temperature-sensitive (rep_{ts}) pSC101 derivative, which carries a mini-Tn10 transposon (miniTn10) with a chloramphenicol-resistance gene (*cam*), the Tn10 transposase gene under control of the lambda P_R promoter and the lambda cl₈₅₇ gene (see Methods). Transposition was induced by a temperature shift from 28 to 42 °C and single miniTn10-Cam^r transposon mutants were selected at 42 °C on chloramphenicol plates and screened for Bgl⁻ and Lac-positive mutants. Strains S764 (b) and S2103 (c) are *lon* mutants that carry an activated *bgl* operon and a fusion of the *bgl* regulatory region to the *lac* operon. The *bgl* operon is activated by a point mutation that improves the CRP-binding site (allele *bgl*-CRP⁺). (b) In strain S764, the *bgl*-*lacZ* fusion carries the same activated *bgl* promoter allele, *bgl*-CRP⁺, as is present at the *bgl* operon. Expression of this *bgl*-*lacZ* fusion is independent of BglG-mediated anti-termination, due to a deletion of the terminator *bgl*-t1 located in the leader of the operon. In addition, the *bgl*G allele *bgl*G^{orf} is present, carrying a mutated translation start codon (Dole *et al.*, 2004a). (c) In strain S2103, the expression of the *bgl*G^{orf}-*lacZ* fusion, which carries the downstream-regulatory site of *bgl*, is directed by the constitutive *lacUV5* promoter. Both of these *lon* mutants are Bgl⁻ and Lac-negative, due to more efficient *bgl* operon silencing by H-NS, whilst the phenotype is Bgl⁻ and Lac-positive in the corresponding wild-type strains [(b), strain S594; (c), strain S2101 (Dole *et al.*, 2004a) and this study].

mutant. This transposon-mutagenesis screen yielded mutations in loci encoding products known to affect *bgl* operon expression, including *leuO*, *yjjQ*-*bglJ*, *rpoS* and *crl*. These mutations increase the *bgl* promoter activity, as shown here for BglJ and LeuO and as known for RpoS and Crl, and thus compensate indirectly for the more efficient downstream repression in the *lon* mutant. In addition, we isolated a suppressor mutant that carries a transposon insertion upstream of the *dnaK* open reading frame, which expresses decreased levels of DnaK. This mutant, which is novel in the context of *bgl*, was found to specifically affect downstream repression by H-NS.

METHODS

Strains, plasmids and media. The genotypes of the *E. coli* strains used in this study are listed in Table 1. All experiments were performed using isogenic strains derived from *E. coli* K-12 strain S541 carrying a deletion of the *bgl* operon and the *lacZ* gene (Dole *et al.*, 2002). Mutations were transduced by using phage T4GT7 (Wilson *et al.*, 1979). Integration of *bgl*-*lacZ* reporter-gene fusions into the chromosomal phage lambda attachment site *attB* was performed as described previously (Diederich *et al.*, 1992; Dole *et al.*, 2002). Plasmids were constructed according to standard techniques (Sambrook & Russell, 2001). Plasmid pKESK18 (see Fig. 1a) is a temperature-sensitive derivative of pSC101 (Hashimoto-Gotoh *et al.*, 1981) with a kanamycin-resistance gene. The plasmid carries the phage lambda cl-857 allele encoding the temperature-sensitive lambda repressor, the Tn10 transposase gene under control of the

phage lambda P_R promoter and a miniTn10 transposon (miniTn10-Cam^r) with a chloramphenicol-resistance gene derived from plasmid pNK2884 (Kleckner *et al.*, 1991). Plasmid pKEAP4 is a pKK223-3 derivative (Brosius & Holy, 1984) that carries a *lacI^r tacOP* cassette followed by a truncated phage T7 gene 10 Shine–Dalgarno sequence [GenBank accession no. NC_001604 (phage T7), positions 22949–22966] to which the *bglI* open reading frame (GenBank no. AE000507, positions 7109–7786) is fused for high expression of BglI. Plasmid pKEAP10 also carries a *lacI^r tacOP* cassette followed by the *leuO* gene with its native translation start (GenBank no. AE000118; positions 625–1786) cloned into a backbone carrying the pBR322 origin of replication and the *bla* gene (Bolivar *et al.*, 1977). Bacteria were grown in LB (Difco), NB (Difco) or minimal M9 (Miller, 1992) media, as indicated. Antibiotics were added to final concentrations of 12 µg tetracycline ml⁻¹, 25 µg kanamycin ml⁻¹, 50 µg ampicillin ml⁻¹, 15 µg chloramphenicol ml⁻¹ and 50 µg spectinomycin ml⁻¹ where necessary.

Transposon mutagenesis. Transposon-mutagenesis screens were performed by using pKESK18 (Fig. 1a) carrying a miniTn10-Cam^r transposon. In this plasmid, replication is temperature-sensitive and, also, expression of the transposase is repressed at 28 °C and induced at 42 °C. Thus, at 28 °C, the plasmid replicates, whilst the transposase is not expressed. Upon a temperature shift, expression of the transposase gene and thus transposition are induced, whilst replication of the plasmid stops, allowing the selection of transposon mutants on chloramphenicol plates at 42 °C. With this system, transposition takes place in approximately 1–5 % of the cells and all of the mutants that we characterized carried single miniTn10-Cam^r transposon insertions. Transformants of strains S594 and S2103 with plasmid pKESK18 were grown at 28 °C in LB medium containing kanamycin and chloramphenicol. To select for transposon mutants, dilutions were plated on MacConkey lactose (Difco)/chloramphenicol plates and incubated at 42 °C. Mutants with a change in the lactose phenotype were restreaked and their Bgl phenotype was tested on bromothymol blue/salicin indicator plates (Dole *et al.*, 2002). In addition, the phenotypes were re-evaluated on M9 minimal plates containing 0.4 % glycerol, 0.1 % tryptone, 0.1 % yeast extract (Difco), 0.2 % Casamino acids and 20 µg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside or 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside ml⁻¹. Of mutants with a double phenotype change, the insertion position of the miniTn10 transposon was determined by sequencing of chromosomal DNA using the miniTn10-specific primer S156 of the sequence 5'-GATGATAAAA-GGCACCTTTGGTCA-3' or by a semi-random, two-step PCR protocol as described by Chun *et al.* (1997). Briefly, in a first, semi-specific PCR, a 'random primer' (S360, 5'-GGCCACGCGTCG-ACTAGTACNNNNNNNNNGATC-3') and a miniTn10-specific primer (S357, 5'-GGCAGGTCGTAAATAGCCGCTTATGT-3', or S358, 5'-CGGTATCAACAGGACACCAGGATTTATTTATTCT-3') were used. The amplification products of this first PCR were reamplified in a second PCR using a primer (S361, 5'-GCTCTAGAGG-CCACGCGTCTGACTAGTAC-3') that matches to the 'random primer' S360 and a nested miniTn10-specific primer (S359, 5'-GCTCTAGAGATCATATGACAAGATGTGTATCCACCTTA-3'). The PCR products were gel-purified and sequenced with primer S359.

β-Galactosidase assays. For enzyme assays, cells were grown in M9 medium containing 1 % (w/v) glycerol, 0.66 % (w/v) Casamino acids (Difco) and 1 µg vitamin B1 ml⁻¹, or in NB medium (Difco), as indicated. Cultures were inoculated to an OD₆₀₀ of 0.1–0.15 from fresh overnight cultures grown in the same medium and grown at 37 or 30 °C as indicated. IPTG was added to this fresh culture, where indicated. Cells were harvested at an OD₆₀₀ of 0.5. The β-galactosidase assays were performed as described previously (Miller,

1992; Dole *et al.*, 2002). The enzyme activities were determined at least three times from at least two independent transformants or integration derivatives. SD values were <10 %.

DnaK Western analysis. Cultures were grown in LB at 30 °C to an OD₆₀₀ of 0.5. IPTG (1 mM) was added where indicated. Cultures were stopped on ice. Then, cells were harvested by centrifugation and resuspended in SDS-PAGE sample buffer (Laemmli, 1970) at an OD₆₀₀ of 0.05 per 10 µl sample buffer. A 5 µl (0.025 OD₆₀₀) aliquot was separated by SDS-PAGE (12 % gel) using an SE600 16 cm gel-electrophoresis unit (GE Healthcare). The gel was blotted onto a 0.45 µm pore size PVDF transfer membrane by using a TE70 semi-dry blotting apparatus (GE Healthcare). The blot was handled using a standard Western blotting protocol (Coligan *et al.*, 2005). Monoclonal mouse antisera directed against DnaK (Stressgen Bioreagents) were used as the primary antibody at 1 µg ml⁻¹; Alexa Fluor 680 rabbit anti-mouse IgG(H+L) (Molecular Probes) was used as the secondary antibody at a concentration of 0.5 µg ml⁻¹. Visualization and quantification were done by using the Odyssey Imaging System (Li-Cor Biosciences) according to the instructions of the manufacturer.

RESULTS

Mutagenesis screen for suppression of more effective downstream repression of *bgl* by H-NS in *lon* mutants

In order to identify factors that suppress the more effective H-NS-mediated downstream repression of *bgl* in a *lon* mutant, we performed a transposon-mutagenesis screen using a modified miniTn10 system (Kleckner *et al.*, 1991) present on the low-copy and replication temperature-sensitive plasmid pKESK18 (Fig. 1a; see Methods for details). To avoid mutations that map *in cis* to the operon, a double-phenotype screening strategy was used (Fig. 1b and c) (Dole *et al.*, 2004a). Both of the *lon* mutant strains S764 and S2103 (containing allele *lon-107::miniTn10-Tc^r*; Table 1), which were screened for suppressor mutations, carry a *bgl* operon allele (*bgl*-CRP⁺) whose promoter is activated by a point mutation improving the CRP-binding site. This *bgl* allele confers a Bgl-positive phenotype in the wild-type, but a Bgl-negative phenotype in the *lon* mutants (Fig. 1b and c) (Dole *et al.*, 2004a). As a second reporter, the *lon* mutants carry *bgl-lacZ* fusions. In one strain (Fig. 1b, S764) the *bgl* promoter, including upstream- and downstream-regulatory elements, was fused to the chromosomal *lac* operon genes (Dole *et al.*, 2004a). In this *bgl-lac* fusion, the same *bgl* promoter allele, *Pbgl*-CRP⁺, is present as at the *bgl* locus. In addition, the terminator *t1* within the leader is deleted ($\Delta t1$) and translation of *bglG* is excluded by mutation of the translation start codon to avoid the necessity for anti-termination by BglG and crosstalk with the *bgl* operon. The second *lon* mutant strain (S2103) carries a *bgl-lacZ* fusion, which is specific for downstream repression by H-NS (Dole *et al.*, 2004b). In this fusion, the *bgl* downstream-regulatory fragment, encompassing the *bglG* allele *bglGorf* (which cannot be translated), is inserted between the constitutive *lacUV5* promoter and the *lacZ* gene (Fig. 1c). Both *bgl-lac* fusions direct a Lac-positive phenotype in the wild-type strains (S594 and S2101), but a

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

Strain	Relevant genotype*	Construction†/reference
BB7049	PA1/lacO <i>dnaKJ</i> <i>lacI</i> ^q	Tomoyasu <i>et al.</i> (1998)
S541	Δ bgl-AC11 Δ lacZ-Y217 <i>ara</i> <i>thi</i> =CSH50 (<i>gpt-lac</i>) ⁺ Δ bgl-AC11 Δ lacZ-Y217	Dole <i>et al.</i> (2002)
S544	<i>bgl</i> -CRP ⁺ Δ lacZ-Y217 <i>ara</i> <i>thi</i>	Dole <i>et al.</i> (2002)
S594	<i>bgl</i> -CRP ⁺ (Bgl ⁺) Δ lacOP::(<i>Spc</i> ^r <i>bgl</i> -CRP ⁺ <i>Pbgl</i> Δ t1 <i>bglGorf</i>) <i>ara</i> <i>thi</i>	Dole <i>et al.</i> (2004a)
S764	S594 <i>lon</i> -107::miniTn10 (II, <i>lon</i> :430)	Dole <i>et al.</i> (2004a)
S1075	S544 <i>lon</i> -107::miniTn10	x T4GT7(S764) Tc ^r
S1553	S541 Δ lon-510 <i>sulA3</i>	Dole <i>et al.</i> (2004a)
S2101	S544 <i>attB</i> ::[<i>Spc</i> ^r <i>lacUV5</i> (+95) <i>bglGorf</i> <i>lacZ</i>]	x pKESD49
S2103	S1075 <i>attB</i> ::[<i>Spc</i> ^r <i>lacUV5</i> (+95) <i>bglGorf</i> <i>lacZ</i>]	x pKESD49
S1729	S764 <i>leuO</i> -Y1::miniTn10-Cam ^r and <i>leuO</i> ⁺ (orientation I: TSD AE000118, 797–805; <i>leuO</i> coding region AE000118, 823–1767); 16 independent isolates, other strains are S1730, S1735, S1741, S1747–S1750, S1752, S1755, S1756 and S1760–S1763	Insertion mutants
S1733	S764 <i>yjjQ/bglJ</i> -Y5::miniTn10-Cam ^r and <i>yjjQ</i> ⁺ / <i>bglJ</i> ⁺ (I: TSD AE000507, 6928–6936; <i>bglJ</i> coding region maps AE000507, 7109–7786); two independent isolates, second strain is S1742	Insertion mutants
S1734	S764 <i>yjjQ/bglJ</i> -Y6::miniTn10-Cam ^r and <i>yjjQ</i> ⁺ / <i>bglJ</i> ⁺ (I: insertion site AE000507, 7072)	Insertion mutant
S1738	S764 <i>dnaKJ</i> -Y17::miniTn10-Cam ^r (I: TSD AE000112, 1562–1570; <i>dnaK</i> coding region AE000507, 1625–3541)	Insertion mutant
S1739	S764 <i>leuO</i> -Y18::miniTn10-Cam ^r and <i>leuO</i> ⁺ (I: TSD AE000118, 684–692)	Insertion mutant
S1740	S764 <i>crl</i> -Y19::miniTn10-Cam ^r (II: TSD AE000132, 4846–4838, <i>crl</i> coding region AE000132, 4545–4946)	Insertion mutant
S1743	S764 <i>crl</i> -Y22::miniTn10-Cam ^r (II: TSD AE000132, 4738–4729)	Insertion mutant
S1751	S764 <i>rpoS</i> -YK30::miniTn10-Cam ^r (I, TSD AE000358: 1556–1564, <i>rpoS</i> coding region AE000358: 2525–1533)	Insertion mutant
S1753	S764 <i>crl</i> -Y32::miniTn10-Cam ^r (II: insertion site AE000132, 4571)	Insertion mutant
S1754	S764 <i>yjjQ/bglJ</i> -Y33::miniTn10-Cam ^r and <i>yjjQ</i> ⁺ / <i>bglJ</i> ⁺ (I: TSD AE000507, 6933–6941)	Insertion mutant
S2142	S2103 <i>dnaKJ</i> -M2::miniTn10-Cam ^r (I: TSD AE000112, 1562–157); two independent isolates, second strain is S2141	Insertion mutants
S2143	S2103 <i>dnaKJ</i> -M3::miniTn10-Cam ^r (I: 29 bp deletion AE000112, 1571–1599)	Insertion mutant
S1213	S541 <i>attB</i> ::[<i>Spc</i> ^r <i>Pbgl</i> (+25) <i>lacZ</i>] (pKEKB30)	Dole <i>et al.</i> (2004b)
S1211	S541 <i>attB</i> ::[<i>Spc</i> ^r <i>Pbgl</i> (–76 to +25) <i>lacZ</i>] (pKEKB25)	Dole <i>et al.</i> (2004b)
S1195	S541 <i>attB</i> ::[<i>Spc</i> ^r <i>PlacUV5</i> (+95) <i>bglGorf</i> <i>lacZ</i>] (pKESD49)	Dole <i>et al.</i> (2004b)
S1556	S1553 <i>attB</i> ::[<i>Spc</i> ^r <i>Pbgl</i> (+25) <i>lacZ</i>] (pKEKB30)	Dole <i>et al.</i> (2004a)
S1554	S1553 <i>attB</i> ::[<i>Spc</i> ^r <i>Pbgl</i> (–76 to +25) <i>lacZ</i>] (pKEKB25)	Dole <i>et al.</i> (2004a)
S1564	S1553 <i>attB</i> ::[<i>Spc</i> ^r <i>PlacUV5</i> (+95) <i>bglGorf</i> <i>lacZ</i>] (pKESD49)	Dole <i>et al.</i> (2004a)
S1773	S1195 <i>leuO</i> -Y1::miniTn10-Cam ^r	x T4GT7(S1729) Cam ^r
S1775	S1213 <i>leuO</i> -Y1::miniTn10-Cam ^r	x T4GT7(S1729) Cam ^r
S1777	S1211 <i>leuO</i> -Y1::miniTn10-Cam ^r	x T4GT7(S1729) Cam ^r
S1787	S1213 <i>yjjQ/bglJ</i> -Y5::miniTn10-Cam ^r	x T4GT7(S1733) Cam ^r
S1797	S1195 <i>yjjQ/bglJ</i> -Y6::miniTn10-Cam ^r	x T4GT7(S1734) Cam ^r
S1799	S1213 <i>yjjQ/bglJ</i> -Y6::miniTn10-Cam ^r	x T4GT7(S1734) Cam ^r
S1801	S1211 <i>yjjQ/bglJ</i> -Y6::miniTn10-Cam ^r	x T4GT7(S1734) Cam ^r
S2712	S1195 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2674	S1213 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2676	S1211 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2708	S1554 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2710	S1556 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2670	S1564 <i>dnaK</i> -M2::miniTn10-Cam ^r	x T4GT7(S2142) Cam ^r
S2888	S1195 PA1/lacO <i>dnaKJ</i> <i>lacI</i> ^q	x T4GT7(BB7049) Cam ^r 1 mM IPTG
S2904	S1213 PA1/lacO <i>dnaKJ</i> <i>lacI</i> ^q	x T4GT7(BB7049) Cam ^r 1 mM IPTG

Table 1. cont.

**P_{bgl}* and *Plac_{UV5}* describe the *bgl* and *lac_{UV5}* promoters, respectively, and *bglGorf* is a *bglG* mutant in which the translation-initiation codon and, in addition, ATG codons 3 and 27 were mutated to GCG (Dole *et al.*, 2004a). For *bgl-lacZ* constructs integrated into *attB*, the relevant structure and the parent plasmid from which these fusions were derived are given. The *bgl* operon allele *bgl-CRP⁺* carries a C to T exchange in the CRP-binding site at position -66 (relative to the transcription start) that causes activation of the *bgl* promoter and, thus, this allele confers a Bgl-positive phenotype. For miniTn10 mutations, orientation I indicates that the *cam* gene is in the same orientation as the mutated gene, whilst in orientation II, it is inserted in the opposite direction. The target site duplication (TSD) or the position of the insertion site is also given. In the latter case, the position of the nucleotide located directly 3' to miniTn10 is given for insertions in orientation I, whilst the position of the nucleotide located directly 5' to miniTn10 is given for insertions in orientation II. All *leuO* and *bglJ* miniTn10 insertion mutants retain a wild-type copy of the respective gene as tested by PCR using gene-specific primers. Cam^r is used for chloramphenicol resistance, Tc^r for tetracycline resistance and Spc^r for spectinomycin resistance.

†Transductions [e.g. x T4GT7(S2142)] and integrations of *bgl-lacZ* reporter constructs (x pKESD49) were performed as described previously (Wilson *et al.*, 1979; Diederich *et al.*, 1992; Dole *et al.*, 2002). For transduction of PA1/*lacO dnaKJ lac^r*, LB/chloramphenicol plates containing 1 mM IPTG were used as selective plates.

Lac-negative phenotype in the *lon* mutant strains S764 and S2103 (Fig. 1b and 1c).

Mutagenesis of the *lon* mutant strain S764 carrying the complete *bgl* regulatory region fused to the *lac* operon yielded in total 25 transposon mutants with a clear double-phenotypic change to Bgl⁺ and Lac⁺. Of these mutants, which were characterized by sequencing of the miniTn10 insertion site, 16 mapped in *leuO*, four mapped in *yjjQ-bglJ*, three in *crl*, one in *rpoS* and one in *dnaK* (Fig. 2a). Interestingly, the second mutagenesis screen for suppressors using the *lon* mutant strain S2103, which carries the *lac_{UV5}-bglGorf-lacZ* reporter specific for downstream repression (Fig. 1c), yielded three insertion mutations with a clear phenotype change to Bgl⁺ and Lac⁺, all of which mapped at the *dnaKJ* locus (Fig. 2b). This result suggests that DnaK (and DnaJ) may affect downstream repression by H-NS, whilst the mutations mapping in *leuO*, *bglJ*, *rpoS* and *crl* may affect the promoter. In agreement with this is the fact that RpoS (together with Crl) is known to repress the *bgl* promoter (Schnetz, 2002). Thus, the miniTn10 insertion mutations mapping in *crl* and *rpoS* are likely to compensate indirectly for the more efficient, H-NS-mediated downstream repression in the *lon* mutant by increasing the promoter activity. The *rpoS* and *crl* mutants were not further analysed. The analysis of the other mutants is presented below. Another interesting result is that no *hns* mutant was isolated, although the *bgl* operon and the *bgl-lacZ* reporter constructs direct a Bgl- and Lac-positive phenotype in a *lon hns* double mutant (Dole *et al.*, 2004a). A similar change in the spectrum of mutations that affect *bgl* was detected before in an *rpoS* background (Moorthy & Mahadevan, 2002).

Constitutive expression of BglJ and LeuO enhances the activity of the *bgl* promoter

All *leuO::miniTn10* insertion mutants map upstream of the *leuO* open reading frame. In 15 of 16 mutants, the insertion site maps 26 bp upstream of an ATG that is equivalent to the translation start characterized for the *Salmonella typhimurium leuO* gene (Chen *et al.*, 2004). In

one mutant (*leuO*-Y18, strain S1739), the insertion site maps 139 bp upstream of *leuO*. Four mutants map within the putative *yjjQ-bglJ* operon at the 3' end of the *yjjQ* open reading frame and upstream of *bglJ*. In allele *yjjQ-bglJ*-Y5 (which was isolated twice), the insertion maps 181 bp upstream of the putative translation start codon of *bglJ*, in allele Y6 it maps 37 bp upstream and in allele Y33 it maps 176 bp upstream. In all of these mutants, the orientation of the miniTn10 transposon may allow constitutive expression of *leuO* and *bglJ*, respectively, by the promoter of the chloramphenicol-resistance gene present within miniTn10-Cam^r. Furthermore, PCR analyses revealed that, in all of these mutants, a copy of the wild-type *leuO* gene and *yjjQ-bglJ* operon is retained (this is not the case in the *rpoS*, *crl* or *dnaK* insertion mutants) (see Discussion).

The insertion mutations isolated at the *bglJ* and *leuO* loci are similar to mutations isolated before. It has also been shown before that constitutive expression of BglJ and LeuO, respectively, relieves silencing of *bgl* (Giel *et al.*, 1996; Ueguchi *et al.*, 1998). Here, we analysed whether the *leuO* and *yjjQ-bglJ* mutations relieve silencing of the *bgl* promoter, downstream silencing by H-NS or both levels of silencing. To this end, we used chromosomally encoded *bgl-lacZ* reporter constructs specific for promoter and downstream repression, respectively (Figs 3 and 4). The expression directed by these *bgl-lacZ* reporter constructs was tested in the wild-type, as well as in *yjjQ-bglJ* and *leuO* mutants, grown to the exponential phase (OD₆₀₀ = 0.5) in minimal M9 glycerol medium.

The expression level of β -galactosidase directed by the *bgl* promoter-*lacZ* reporter (which carries the promoter fused at position +25 to *lacZ*) increased from 74 units in the wild-type to 225 units in the *bglJ*-Y5 mutant and to 675 units in the *bglJ*-Y6 mutant, demonstrating that, presumably, constitutive expression of *bglJ* activates the *bgl* promoter. The effect observed with allele *yjjQ-bglJ*-Y6 is bigger, possibly because the insertion element maps closer to *bglJ* (37 bp upstream). Likewise, in the *leuO*-Y1 mutant, the *bgl* promoter activity increased to 215 units, which suggests that

Fig. 2. Mutants that suppress efficient silencing of *bgl* in *lon* mutants. The insertion sites of miniTn10-Cam^r mutations causing suppression of more efficient silencing of *bgl* in the *lon* mutant are indicated by arrowheads. (a) Suppressor mutants that increase expression of *bgl* and the *bgl*-*lacZ* fusion in strain S764 (Fig. 1b) map in *leuO* (16 mutants), *yjiQ*-*bglJ* (four mutants), *crl* (three mutants), *rpoS* (one mutant) and *dnaK* (one mutant). In the *leuO*, *bglJ* and *dnaK* mutants, the miniTn10-Cam^r insertion maps 5' to the coding region and is thus likely to direct constitutive expression of these genes. In contrast, the miniTn10-Cam^r insertions mapping in *crl* and at the 3' end of *rpoS* disrupt the coding region of these genes. (b) Three suppressor mutants were isolated that increase expression of the *bgl* and the *lacUV5*-*bglGorf*-*lacZ* fusion (strain S2103, Fig. 1c). All three mutants map 5' to the *dnaK* coding region (alleles M1, M2 and M3). One of them (allele *dnaK*-M3) is associated with a 29 bp deletion (indicated by an open arrowhead). (a, b) The sequence position of the miniTn10-Cam^r insertion sites of all mutants is given in Table 1: *leuO* mutants include strain S1729 and its identical isolates S1730, S1735, S1741, S1747-S1750, S1752, S1755-S1756 and S1760-S1763, as well as strain S1739; *yjiQ*-*bglJ* mutants include the identical strains S1733 and S1742, as well as strains S1734 and S1754; the *crl* mutants are strains S1740, S1743 and S1753; the *rpoS* mutant is strain S1751; and the *dnaK* mutants are strain S1738, as well as strains S2141, S2142 and S2143, which were isolated in the second screen (Fig. 1c). All *leuO* and *yjiQ*-*bglJ* miniTn10 insertion mutants retain a copy of the wild-type gene in addition to the mutant allele. This is not the case in the *rpoS*, *crl* or *dnaK* miniTn10 mutants.

LeuO also activates the *bgl* promoter (Fig. 3a). In addition, the *bglJ* and *leuO* genes were cloned under the control of the IPTG-inducible *tac* promoter in the pBR-derived plasmids pKEAP4 and pKEAP10 (see Methods for details). These plasmids were used for transformation of the wild-type strain carrying the *bgl* promoter-*lacZ* fusion. Induction of *bglJ* expression with IPTG resulted in an increase of

β -galactosidase expression directed by the *bgl* promoter to 570 units, whilst induction of *leuO* expression caused an increase to 735 units (Fig. 3a), confirming that BglJ and LeuO are activators of the *bgl* promoter and that the miniTn10 insertions cause constitutive expression of these genes. A *bgl* promoter allele (ΔP_{bgl}) that lacks the upstream silencer sequence necessary for H-NS-mediated repression was also tested. The activity of this promoter (ΔP_{bgl}) is approximately fourfold higher than that of the wild-type promoter (Dole *et al.*, 2004b) (also compare Fig. 3a and b, 74 versus 300 units). The *bglJ*-Y6 mutation had no effect on the ΔP_{bgl} promoter (320 units compared with 300 units in the wild-type), whilst the *leuO*-Y1 mutations caused a rather moderate 1.4-fold increase (to 425 units). These data suggest that constitutive expression of BglJ and LeuO activates the *bgl* promoter by counteracting the H-NS-mediated repression of the promoter.

The *bgl*-*lacZ* downstream reporter construct (which carries the *bglGorf* downstream regulatory region inserted between the *lacUV5* promoter and *lacZ* as described above) was also analysed in the wild-type and the *bglJ*-Y6 and *leuO*-Y1 mutants. The β -galactosidase level directed by the downstream reporter (Fig. 4a) did not vary significantly between the wild-type (62 units), the *yjiQ*-*bglJ*-Y6 mutant (69 units) and the *leuO*-Y1 mutant (75 units), demonstrating that LeuO and BglJ have no impact on downstream repression by H-NS. Thus, suppression of the more effective H-NS-mediated downstream repression in the *lon* mutant by expression of *bglJ* and *leuO* is indirect and compensated for by an increased promoter activity.

In the *dnaKJ*::miniTn10 mutant, downstream repression by H-NS is specifically suppressed

In both screening strategies (Fig. 1), insertion mutations mapping at the *dnaKJ* locus were isolated (Fig. 2). The single *dnaKJ*::miniTn10 mutant isolated in the first screen is identical to two of the three mutants isolated in the second screen, which was performed in the strain carrying the *bgl*-*lacZ* downstream reporter. These mutants carry a miniTn10 insertion that disrupts the *dnaK* promoter and that maps 63 bp upstream of the *dnaK* ATG start codon. In the other insertion mutation, miniTn10 also maps upstream of the *dnaK* open reading frame (26 bp upstream of the ATG) but, in addition, the mutant carries a small deletion of 29 bp. The phenotype of this latter mutant is weaker and this mutant was not further characterized.

The possible role of DnaKJ in *bgl* promoter and *bgl* downstream repression by H-NS and the modulation of the latter by Lon were addressed by using the same approach as described above for the *leuO* and *bglJ* mutants, i.e. by using *bgl* promoter and *bgl* downstream *lacZ* reporter constructs. The *dnaKJ*::miniTn10 mutants were grown in NB medium, because the mutants were also analysed in combination with *lon*, and *lon* mutants grow poorly in minimal medium.

Fig. 3. The activity of the *bgl* promoter increases upon overexpression of BglJ or LeuO, but not in *dnaKJ* mutants. The wild-type *bgl* promoter (encompassing positions -450 to $+25$) (a, c) and a *bgl* promoter lacking the upstream silencer (ΔP_{bgl} , encompassing positions -76 to $+25$) (b, d) were fused to *lacZ*, and these reporter constructs were integrated into the chromosomal *attB* site. β -Galactosidase activities shown were determined for cultures grown to $OD_{600}=0.5$ in minimal M9 glycerol medium (a, b) or in NB medium (c, d). (a) In the wild-type (strain S1213), the *bgl* promoter directs 74 units of β -galactosidase activity. In the *yjiQ*-*bglJ* mutants, the promoter directs 225 units (allele *bglJ*-Y5, strain S1787) and 675 units (allele *bglJ*-Y6, strain S1799) of β -galactosidase activity. In the *leuO*-Y1 mutant (strain S1776), 215 units of β -galactosidase activity were detected. Upon overexpression of BglJ (+BglJ) and LeuO (+LeuO), the promoter activity increased to 570 and 735 units, respectively. Overexpression of BglJ and LeuO was accomplished by using plasmids pKEAP4 (BglJ) and pKEAP10 (LeuO), which carry the *bglJ* and *leuO* genes, respectively, under control of the inducible *tac* promoter. For induction of *bglJ* and *leuO* expression, 1 mM IPTG was added to the exponentially growing cultures. (b) Activity of the *bgl* promoter lacking the upstream silencer varied only slightly, from 300 units in the wild-type (strain S2111) to 320 units in the *bglJ*-Y6 mutant (strain S1801) and to 425 units in the *leuO*-Y1 mutant (strain S1777). (c, d) The activity of the promoter constructs did not differ significantly in the *dnaK*::miniTn10-Cam^r mutant. (c) When cells were grown in NB medium, the wild-type promoter directed 140 units in the wild-type strain (S1213), 165 units in the *dnaK* mutant (S2674), 135 units in the *lon* mutant (S1556) and 145 units in the *lon dnaK* double mutant (S2710). (d) The promoter lacking the upstream silencer directed 470 units in the wild-type background (S1211), 525 units in the *dnaK* mutant (S2676), 520 in the *lon* mutant (S1554) and 450 units in the *lon dnaK* double mutant (S2708). (e) The promoter activity was not affected in a PA1/*lacO*1 *dnaKJ* strain grown without or with 1 mM IPTG in LB medium at 30 °C. The promoter directed 80 units in the wild-type (S1213), 335 units in the *dnaKJ*::miniTn10 mutant (S2674), 90 units in the PA1/*lacO*1 *dnaKJ* strain (S2904) when grown without IPTG (–) and 75 units when grown with 1 mM IPTG (+).

First, we analysed whether the *dnaKJ*::miniTn10 mutant (allele *dnaKJ*-M2) affects the *bgl* promoter activity by using the *bgl* promoter–*lacZ* reporter described above (Fig. 3c). In the wild-type, this reporter directs the expression of 140 units of β -galactosidase activity when cells are grown in NB. In the *dnaKJ*::miniTn10 mutant, 165 units were detected, i.e. the promoter activity was not changed significantly. Likewise, the promoter activity remained unaffected in a *lon* mutant (Dole *et al.*, 2004a) and a *lon dnaKJ*::miniTn10 double mutant (Fig. 3c). The *bgl* promoter allele (ΔP_{bgl}) that lacks the upstream silencer sequence necessary for repression by H-NS was likewise not affected by the mutation of *dnaKJ* (Fig. 3d). Thus DnaK (and DnaJ) have no impact on the activity of the *bgl* promoter or its repression by H-NS.

In contrast, the expression level of β -galactosidase directed by the *bgl*–*lacZ* downstream reporter, which decreases from

95 units in the wild-type to 40 units in the *lon* mutant (Fig. 4b) (Dole *et al.*, 2004a), was affected by DnaK. In the *dnaKJ*::miniTn10 mutant, the expression level increased to 220 units, i.e. approximately twofold compared with the wild-type, and in the *lon dnaKJ*::miniTn10 double mutant, the expression level increased to 155 units, i.e. approximately fourfold compared with the *lon* single mutant (40 units). These data show that DnaK (and possibly DnaJ) specifically affect the downstream repression of *bgl* by H-NS and its modulation by Lon.

DnaK is required for efficient downstream silencing by H-NS

In the *dnaKJ*::miniTn10 mutant, the miniTn10 insertion maps upstream of the *dnaK* open reading frame. Therefore, these insertions may direct constitutive expression of the *dnaKJ* operon by the promoter of the

Fig. 4. In *dnaK* mutants, downstream repression of *bgl* by H-NS is specifically reduced, whilst BglI and LeuO have no impact. The role of LeuO and BglI (a), as well as DnaK and Lon, on downstream repression of *bgl* was tested by using a *bgl-lacZ* downstream reporter construct that carries the *bglG* coding region, *bglGorf*, inserted between the *lacUV5* promoter and the *lacZ* gene integrated into the chromosomal *attB* site. β -Galactosidase activities shown were determined for cultures grown to $OD_{600}=0.5$ in minimal M9 glycerol medium at 37 °C (a), NB medium at 37 °C (b) or LB medium at 30 °C (c). (a) In the wild-type (strain S1195), 62 units of β -galactosidase activity were measured, which was not changed significantly in the *bglJ-Y6* mutant (strain S1797) and the *leuO-Y1* mutant (strain S1773). (b) Wild-type cells (strain S1195) grown in NB medium expressed 95 units of β -galactosidase activity. In the *dnaKJ* mutant (strain S2712), the expression level increased by approximately twofold to 220 units. In the *lon* mutant (strain S1564), 40 units were detected, whilst in the *dnaKJ lon* double mutant (strain S2670), the expression level increased by approximately fourfold to 155 units. (c) When cells were grown at 30 °C in LB medium, 155 units were detected in the wild-type (S1195) and 335 units in the *dnaKJ::miniTn10* mutant (S2712). In the *PA1/lacO dnaKJ* strain (S2888), the expression level was increased to 415 units when IPTG was omitted, and decreased to 76 units when *dnaKJ* expression was induced with IPTG.

chloramphenicol-resistance gene located within the mini-Tn10 transposon. To analyse whether an increase or a decrease in the cellular DnaK level affects downstream repression by H-NS, an additional *dnaKJ* mutant was used, in which the *dnaKJ* promoter is replaced by a *lacI* *PA1/lacO*

cassette (Tomoyasu *et al.*, 1998). Thus, *dnaKJ* expression requires IPTG and DnaKJ levels are very low when cells are grown without IPTG. Downstream repression of *bgl* by H-NS was tested in the *PA1/lacO dnaKJ* strain using the downstream reporter (*lacUV5-bglGorf-lacZ*) as described above. As these cultures had to be grown in LB at 30 °C when grown without IPTG, the β -galactosidase assay for the wild-type and the *dnaKJ::miniTn10* mutant was repeated at these conditions (Fig. 4c). In the wild-type, 156 units of β -galactosidase activity were detected and the activity increased to 335 units in the *dnaKJ::miniTn10* mutant. In the *PA1/lacO dnaKJ* strain, 76 units of β -galactosidase activity were detected when IPTG for induction of the *pA1/lacO1* promoter was present, i.e. high DnaKJ levels support downstream repression. In contrast, the expression level increased to 415 units when IPTG for induction of *dnaKJ* was omitted. This effect was specific for downstream repression. The activity of the *Pbgl-lacZ* promoter construct did not vary in the *PA1/lacO dnaKJ* strain when grown with or without IPTG (Fig. 3e). These data indicate that expression of *dnaKJ* is required for downstream repression by H-NS. To further strengthen this argument, the DnaK levels were analysed in a Western blot by using DnaK-specific antibodies (Fig. 5). The quantitative Western analysis demonstrated that DnaK levels are reduced by approximately twofold in the *dnaKJ::miniTn10* mutant compared with the wild-type. In the *PA1/lacO dnaKJ* strain, DnaK levels were very low (fivefold lower than the wild-type) when cells were grown without IPTG, whilst the DnaK protein level was much higher upon induction with IPTG than in the wild-type strain (17-fold increase) (Fig. 5). Thus, the cellular DnaK protein levels correlate well with the differences in downstream repression of *bgl* by H-NS (Fig. 4c), i.e. downstream repression is more effective when DnaKJ is present.

Fig. 5. The DnaK level is lower in the *dnaKJ::miniTn10*-Cam^r mutant. A quantitative Western blot analysis of DnaK protein levels expressed in the wild-type (S1195), the *dnaKJ::miniTn10* mutant (allele *dnaKJ-M2*; strain S2712) and the *PA1/lacO dnaKJ* strain (S2888) grown without IPTG (-IPTG) and with IPTG (+IPTG) at 30 °C in LB medium to $OD_{600}=0.5$ was performed. The Western blot and the result of the quantitative analysis given as peak intensity, as well as relative protein levels compared with the wild-type (wt; set as 100 arbitrary units), are shown.

DISCUSSION

Silencing of *bgl* by H-NS occurs through binding of H-NS to two sites, located upstream and downstream of the promoter. The latter level of repression by H-NS also affects transcription elongation (Dole *et al.*, 2004b). Here, we have shown that this downstream repression is modulated by DnaK, an HSP70 family chaperone (Dougan *et al.*, 2002), and possibly DnaJ, the DnaK co-chaperone. Lon protease also affects the downstream repression (Dole *et al.*, 2004a). DnaKJ (as Lon) has no impact on silencing of the promoter. Furthermore, we have shown that constitutive expression of transcription factors LeuO and BglJ relieves silencing of the *bgl* operon by counteracting repression of the promoter by H-NS through the upstream site, whilst BglJ and LeuO do not affect downstream repression. These data demonstrate that upstream and downstream repression of *bgl* by H-NS is regulated independently by LeuO and BglJ, as well as DnaKJ. Although upstream and downstream repression can be separated, silencing of *bgl* is significantly more effective when both silencers are present, indicating cooperativity between the two sites, as shown previously (Dole *et al.*, 2004b).

Mutations characterized so far that relieve silencing (or repression) of the *bgl* operon by H-NS affect only one level of repression, the repression of the *bgl* promoter. Here, we have shown that the two transcription factors LeuO and BglJ also counteract the H-NS-mediated repression of the promoter. As, under laboratory growth conditions, the *bgl* operon is always repressed, the expression level of *leuO* and *bglJ* may be too low for activation of *bgl*. The regulation of *bglJ* has not been studied to date. Preliminary data from our lab indicate that it is repressed by H-NS. The expression of *leuO* is likewise repressed by H-NS, and not detectable during exponential growth (Klauck *et al.*, 1997; Chen *et al.*, 2004). However, *leuO* expression is induced in a ppGpp-dependent manner by starvation for branched-chain amino acids, and LeuO is necessary for resuming growth after such a starvation (Fang *et al.*, 2000; Majumder *et al.*, 2001). It is not known whether such a transient induction of *leuO* is sufficient to relieve silencing of *bgl*. The miniTn10 insertion mutants isolated in this work are similar to Tn10 mutants isolated before in the context of *bgl* (Giel *et al.*, 1996; Ueguchi *et al.*, 1998) and apparently direct constitutive expression of *bglJ* and *leuO*, respectively. Constitutive expression of *leuO* and *bglJ* results in activation of the *bgl* promoter. This explains the dominance of the *bglJ* and *leuO* mutants over the wild-type copy of the *bglJ* and *leuO* gene, respectively, also present in the strains, as they are repressed by H-NS. Similar *leuO::Tn10* transposon mutants to those isolated here were isolated in different contexts and shown to enhance expression of *cadA*, encoding an acid-inducible lysine decarboxylase, as well as to reduce RpoS levels (Shi & Bennett, 1994; Klauck *et al.*, 1997). The regulation of RpoS levels by LeuO is indirect and mediated through the small regulatory RNA DsrA, which, at low temperature, enhances translation of the *rpoS* mRNA and represses translation of the *hns* mRNA (Klauck *et al.*, 1997; Lease & Belfort, 2000;

Repoila & Gottesman, 2003). Thus, LeuO belongs to a regulatory network that involves RpoS, H-NS, Hfq and DsrA (Klauck *et al.*, 1997; Repoila & Gottesman, 2003), and the *bgl* operon is clearly a system that is controlled by this network.

The second, downstream level of *bgl* operon repression by H-NS is not affected by LeuO, BglJ or RpoS/Crl. However, this level of repression is modulated by Lon (Dole *et al.*, 2004a). In Lon-deficient mutants, the repression is more effective. This effect is independent of the H-NS homologue StpA (Dole *et al.*, 2004a), a Lon target protein (Johansson & Uhlin, 1999; Johansson *et al.*, 2001). In addition, as shown here, downstream repression is modulated by the heat shock-induced DnaKJ chaperone system. The *dnaKJ* mutants carry a miniTn10 insertion upstream of the *dnaK* open reading frame. Western analysis revealed that this mutant expresses lower levels of DnaK than the wild-type. Likewise, downstream repression is inefficient in a PA1/*lacO dnaKJ* strain when expression of *dnaKJ* is not induced with IPTG. These data imply that DnaKJ is required for efficient downstream repression, which indicates a modulation of H-NS activity by chaperones. Interestingly, the mutation of an HSP66 protein and DnaK homologue encoded by *hscA* was also found to have some effects on silencing by H-NS (Kawula & Lelivelt, 1994). Presently, it is not known whether Lon and the DnaKJ chaperone system affect downstream repression of *bgl* by H-NS directly or indirectly. One speculative possibility is that Lon and DnaKJ modulate the H-NS repressing complex or change the level of *hns* expression. However, Lon and DnaKJ specifically modulate downstream repression by H-NS, but not *bgl* promoter repression. In addition, we found no role for DnaKJ in silencing of *proU* by H-NS (data not shown). Therefore, Lon and DnaKJ may affect an additional process that is specific for downstream repression, e.g. modulation of the elongating RNA polymerase complex and its translocation along the DNA.

Results presented here are in agreement with and extend the finding that repression of the *bgl* operon is linked tightly to the regulatory network controlling the stress response in *E. coli*. Strictly controlled repression of *bgl* supports the idea that the utilization of aryl β -D-glucosides is deleterious under certain conditions (Reynolds *et al.*, 1981), whilst conservation of silencing of the *bgl* operon that is present in a majority of *E. coli* strains (G. Neelakanta and K. Schnetz, unpublished data) suggests an important role for *bgl* under specific conditions in nature.

ACKNOWLEDGEMENTS

We thank Sandra Malcher for excellent technical assistance and Sudhanshu Dole for suggestions. The work was funded by the Deutsche Forschungsgemeinschaft through grant Schn 371/8 and the International Graduate School of Genetics and Functional Genomics at the University of Cologne.

REFERENCES

- Amster-Choder, O. & Wright, A. (1993). Transcriptional regulation of the *bgl* operon of *Escherichia coli* involves phosphotransferase system-mediated phosphorylation of a transcriptional antiterminator. *J Cell Biochem* **51**, 83–90.
- Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L. & Boyer, H. W. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2**, 95–113.
- Brosius, J. & Holy, A. (1984). Regulation of ribosomal RNA promoters with a synthetic *lac* operator. *Proc Natl Acad Sci U S A* **81**, 6929–6933.
- Chen, C.-C., Chou, M.-Y., Huang, C.-H., Majumder, A. & Wu, H.-Y. (2004). A cis-spreading nucleoprotein filament is responsible for the gene silencing activity found in the promoter relay mechanism. *J Biol Chem* **280**, 5101–5112.
- Chun, K. T., Edenberg, H. J., Kelley, M. R. & Goebel, M. G. (1997). Rapid amplification of uncharacterized transposon-tagged DNA sequences from genomic DNA. *Yeast* **13**, 233–240.
- Coligan, J. E., Dunn, B. M., Ploegh, H. L., Speicher, D. W. & Wingfield, P. T. (2005). *Current Protocols in Protein Science*. Chichester and New York: Wiley. doi:10.1002/0471140864.ps0024
- Dame, R. T., Wyman, C., Wurm, R., Wagner, R. & Goosen, N. (2002). Structural basis for H-NS-mediated trapping of RNA polymerase in the open initiation complex at the *rrnB* P1. *J Biol Chem* **277**, 2146–2150.
- Diederich, L., Rasmussen, L. J. & Messer, W. (1992). New cloning vectors for integration into the lambda attachment site *attB* of the *Escherichia coli* chromosome. *Plasmid* **28**, 14–24.
- Dole, S., Kühn, S. & Schnetz, K. (2002). Post-transcriptional enhancement of *Escherichia coli* *bgl* operon silencing by limitation of BglG-mediated antitermination at low transcription rates. *Mol Microbiol* **43**, 217–226.
- Dole, S., Klinge, Y., Nagarajavel, V. & Schnetz, K. (2004a). The protease Lon and the RNA-binding protein Hfq reduce silencing of the *Escherichia coli* *bgl* operon by H-NS. *J Bacteriol* **186**, 2708–2716.
- Dole, S., Nagarajavel, V. & Schnetz, K. (2004b). The histone-like nucleoid structuring protein H-NS represses the *Escherichia coli* *bgl* operon downstream of the promoter. *Mol Microbiol* **52**, 589–600.
- Dorman, C. J. (2004). H-NS: a universal regulator for a dynamic genome. *Nat Rev Microbiol* **2**, 391–400.
- Dougan, D. A., Mogk, A. & Bukau, B. (2002). Protein folding and degradation in bacteria: to degrade or not to degrade? That is the question. *Cell Mol Life Sci* **59**, 1607–1616.
- Fang, M., Majumder, A., Tsai, K.-J. & Wu, H.-Y. (2000). ppGpp-dependent *leuO* expression in bacteria under stress. *Biochem Biophys Res Commun* **276**, 64–70.
- Giel, M., Desnoyer, M. & Lopilato, J. (1996). A mutation in a new gene, *bglJ*, activates the *bgl* operon in *Escherichia coli* K-12. *Genetics* **143**, 627–635.
- Görke, B. (2003). Regulation of the *Escherichia coli* antiterminator protein BglG by phosphorylation at multiple sites and evidence for transfer of phosphoryl groups between monomers. *J Biol Chem* **278**, 46219–46229.
- Gottesman, S. (1996). Proteases and their targets in *Escherichia coli*. *Annu Rev Genet* **30**, 465–506.
- Hashimoto-Gotoh, T., Franklin, F. C. H., Nordheim, A. & Timmis, K. N. (1981). Specific-purpose plasmid cloning vectors. I. Low copy number, temperature-sensitive, mobilization-defective pSC101-derived containment vectors. *Gene* **16**, 227–235.
- Homma, F., Krin, E., Laurent-Winter, C., Soutourina, O., Malpertuy, A., Le Caer, J.-P., Danchin, A. & Bertin, P. (2001). Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. *Mol Microbiol* **40**, 20–36.
- Johansson, J. & Uhlin, B. E. (1999). Differential protease-mediated turnover of H-NS and StpA revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. *Proc Natl Acad Sci U S A* **96**, 10776–10781.
- Johansson, J., Eriksson, S., Sonden, B., Wai, S. N. & Uhlin, B. E. (2001). Heteromeric interactions among nucleoid-associated bacterial proteins: localization of StpA-stabilizing regions in H-NS of *Escherichia coli*. *J Bacteriol* **183**, 2343–2347.
- Kajitani, M. & Ishihama, A. (1991). Identification and sequence determination of the host factor gene for bacteriophage Q β . *Nucleic Acids Res* **19**, 1063–1066.
- Kawula, T. H. & Lelivelt, M. J. (1994). Mutations in a gene encoding a new Hsp70 suppress rapid DNA inversion and *bgl* activation, but not *proU* derepression in *hns-1* mutant *Escherichia coli*. *J Bacteriol* **176**, 610–619.
- Klauck, E., Böhringer, J. & Hengge-Aronis, R. (1997). The LysR-like regulator LeuO in *Escherichia coli* is involved in the translational regulation of *rpoS* by affecting the expression of the small regulatory DsrA-RNA. *Mol Microbiol* **25**, 559–569.
- Kleckner, N., Bender, J. & Gottesman, S. (1991). Uses of transposons with emphasis on Tn10. *Methods Enzymol* **204**, 139–180.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Lease, R. A. & Belfort, M. (2000). Riboregulation by DsrA RNA: *trans*-actions for global economy. *Mol Microbiol* **38**, 667–672.
- Lopilato, J. & Wright, A. (1990). Mechanisms of activation of the cryptic *bgl* operon of *Escherichia coli* K-12. In *The Bacterial Chromosome*, pp. 435–444. Edited by K. Drlica & M. Riley. Washington, DC: American Society for Microbiology.
- Majumder, A., Fang, M., Tsai, K.-J., Ueguchi, C., Mizuno, T. & Wu, H.-Y. (2001). LeuO expression in response to starvation for branched-chain amino acids. *J Biol Chem* **276**, 19046–19051.
- Miller, J. H. (1992). *A Short Course in Bacterial Genetics: a Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Moorthy, S. & Mahadevan, S. (2002). Differential spectrum of mutations that activate the *Escherichia coli* *bgl* operon in an *rpoS* genetic background. *J Bacteriol* **184**, 4033–4038.
- Mukerji, M. & Mahadevan, S. (1997). Characterization of the negative elements involved in silencing the *bgl* operon of *Escherichia coli*: possible roles for DNA gyrase, H-NS, and CRP-cAMP in regulation. *Mol Microbiol* **24**, 617–627.
- Nieto, J. M., Madrid, C., Prenafeta, A., Miquel, E., Balsalobre, C., Carrascal, M. & Juárez, A. (2000). Expression of the hemolysin operon in *Escherichia coli* is modulated by a nucleoid-protein complex that includes the proteins Hha and H-NS. *Mol Gen Genet* **263**, 349–358.
- Prasad, I. & Schaeffer, S. (1974). Regulation of the β -glucoside system in *Escherichia coli* K-12. *J Bacteriol* **120**, 638–650.
- Prosseda, G., Falconi, M., Giangrossi, M., Gualerzi, C. O., Micheli, G. & Colonna, B. (2004). The *virF* promoter in *Shigella*: more than just a curved DNA stretch. *Mol Microbiol* **51**, 523–537.
- Repoila, F. & Gottesman, S. (2003). Temperature sensing by the *dsrA* promoter. *J Bacteriol* **185**, 6609–6614.
- Reusch, R. N., Shabalin, O., Crumbaugh, A., Wagner, R., Schröder, O. & Wurm, R. (2002). Posttranslational modification of *E. coli*

histone-like protein H-NS and bovine histones by short-chain poly-(R)-3-hydroxybutyrate (cPHB). *FEBS Lett* **527**, 319–322.

Reynolds, A. E., Felton, J. & Wright, A. (1981). Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K12. *Nature* **293**, 625–629.

Reynolds, A. E., Mahadevan, S., LeGrice, S. F. J. & Wright, A. (1986). Enhancement of bacterial gene expression by insertion elements or by mutation in a CAP-cAMP binding site. *J Mol Biol* **191**, 85–95.

Sambrook, J. & Russell, D. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Schnetz, K. (1995). Silencing of *Escherichia coli bgl* promoter by flanking sequence elements. *EMBO J* **14**, 2545–2550.

Schnetz, K. (2002). Silencing of the *Escherichia coli bgl* operon by RpoS requires Crl. *Microbiology* **148**, 2573–2578.

Schnetz, K. & Rak, B. (1988). Regulation of the *bgl* operon of *Escherichia coli* by transcriptional antitermination. *EMBO J* **7**, 3271–3277.

Schnetz, K. & Wang, J. C. (1996). Silencing of the *Escherichia coli bgl* promoter: effects of template supercoiling and cell extracts on promoter activity *in vitro*. *Nucleic Acids Res* **24**, 2422–2429.

Schröder, O. & Wagner, R. (2002). The bacterial regulatory protein H-NS – a versatile modulator of nucleic acid structures. *Biol Chem* **383**, 945–960.

Shi, X. & Bennett, G. N. (1994). Plasmids bearing *hfq* and the *hns*-like gene *stpA* complement *hns* mutants in modulating arginine decarboxylase gene expression in *Escherichia coli*. *J Bacteriol* **176**, 6769–6775.

Singh, J., Mukerji, M. & Mahadevan, S. (1995). Transcriptional activation of the *Escherichia coli bgl* operon: negative regulation by DNA structural elements near the promoter. *Mol Microbiol* **17**, 1085–1092.

Tomoyasu, T., Ogura, T., Tatsuta, T. & Bukau, B. (1998). Levels of DnaK and DnaJ provide tight control of heat shock gene expression and protein repair in *Escherichia coli*. *Mol Microbiol* **30**, 567–581.

Ueguchi, C., Ohta, T., Seto, C., Suzuki, T. & Mizuno, T. (1998). The *leuO* gene product has a latent ability to relieve *bgl* silencing in *Escherichia coli*. *J Bacteriol* **180**, 190–193.

Ussery, D. W., Hinton, J. C. D., Jordi, B. J. A. M. & 7 other authors (1994). The chromatin-associated protein H-NS. *Biochimie* **76**, 968–980.

Williams, R. M., Rimsky, S. & Buc, H. (1996). Probing the structure, function, and interactions of the *Escherichia coli* H-NS and StpA proteins by using dominant negative derivatives. *J Bacteriol* **178**, 4335–4343.

Wilson, G. G., Young, K. Y. K., Edlin, G. J. & Konigsberg, W. (1979). High-frequency generalised transduction by bacteriophage T4. *Nature* **280**, 80–82.