A Q-like transcription factor regulates biofilm development in *Escherichia coli* by controlling expression of the DLP12 lysis cassette

Karl-Gustav Rueggeberg,¹ Faustino A. Toba,¹ Mitchell G. Thompson,¹ Bryan R. Campbell¹ and Anthony G. Hay¹,²

¹Department of Microbiology, Cornell University, Ithaca, NY 14853, USA
²Institute for Comparative and Environmental Toxicology, Cornell University, Ithaca, NY 14853, USA

The DLP12 lysis cassette (essD, ybcT,  czpDrizoD) is required in certain *Escherichia coli* strains for normal curli expression and biofilm development. Tightly controlled regulation of the lysis cassette is of particular importance, since its overexpression causes host cell lysis. *In silico* analysis revealed a putative intrinsic transcriptional terminator 100 bp upstream of essD and within 2000 bp of ybcQ (QDLP12), a putative lambda (λ) Q-like antiterminator. We hypothesized that QDLP12 may be required for effective expression of the lysis cassette. In this work we report on the role of QDLP12 as a positive regulator of DLP12 lysis cassette expression. Mutants lacking QDLP12 exhibited a biofilm-defective phenotype analogous to that of the lysis cassette knockouts. This defect occurred through the downregulation of curli transcription, which is also consistent with that seen in the lysis cassette mutants and was restored by complementation by ectopic expression of QDLP12. In addition, QDLP12 overexpression caused cell lysis, as demonstrated by leakage of β-galactosidase activity from cells. This was accompanied by upregulation of the DLP12 lysis cassette as demonstrated by increased essD transcription, which was documented with gfp-reporter assays, RT-PCR and chromatin immunoprecipitation (ChIP). We provide evidence that this Q-mediated effect resulted from direct interaction of QDLP12 with the lysis cassette promoter (essDp), as demonstrated by electrophoretic gel mobility shift assay (EMSA). We propose that QDLP12 encodes a functional transcriptional regulator, which promotes expression of the DLP12 lysis cassette. This work provides evidence of a regulator from a defective prophage affecting host cell physiology.

INTRODUCTION

Biofilm-forming bacteria can colonize biotic and abiotic surfaces. Initial attachment is mediated by extracellular polysaccharides and monomeric proteins, or by more structured components such as pili, flagella and curli (Sheikh et al., 2001; Vidal et al., 1998). Once adhered, the bacteria adopt a multicellular lifestyle through the formation of microcolonies, which later grow into a mature biofilm. The bacteria within these biofilms are more resistant to environmental insults such as nutrient starvation, desiccation and antibiotics (Karatan & Watnick, 2009; Sauer et al., 2002; Stewart & Franklin, 2008; Stoodley et al., 2002).

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Curli, in particular, have been implicated in the initial adhesion phase which leads to biofilm formation in several strains of *Escherichia coli* (Fink et al., 2012; Lee et al., 2011). In most *E. coli* the biofilm master regulator csgD is critical for modulating curli production and serves as a hub for integrating various extracellular signals, thereby dictating whether or not the bacteria initiate biofilm formation (Ogasawara et al., 2010, 2011; Pesavento et al., 2008). Recently, several groups have demonstrated that, in addition to its regulation by over a dozen transcription factors (Boehm & Vogel, 2012), CsgD expression is also regulated post-transcriptionally. This latter effect is mediated by at least five known small RNAs, which bind to the 5′ untranslated region of the csgD transcript, preventing ribosome binding and/or enhancing transcript degradation (Boehm & Vogel, 2012; Holmqvist et al., 2010; Jørgensen et al., 2012; Mika et al., 2012; Thomason et al., 2012). This diverse range of mechanisms permits subtle physiological changes to greatly alter curli production.

In addition to the genes encoding surface structures, early transcriptional studies demonstrated that bacteriophage
genes were highly upregulated within Gram-positive and Gram-negative bacteria in biofilms (Stanley et al., 2003; Whiteley et al., 2001). The importance of prophages was confirmed by the deletion of Pφ4 and Pφ1 prophages in Pseudomonas aeruginosa, resulting in defects in the maturation and dispersal of biofilms, respectively. E. coli K-12 biofilms have been shown to be dramatically affected by the deletion of specific prophages, with the loss of a cryptic prophage CP4-57 dramatically increasing biofilm formation, whereas the loss of others decreased it (Wang et al., 2010).

In E. coli, lambdoid prophage genes such as bor and lom had previously been linked to resistance to animal sera and increased surface adhesion (Canchaya et al., 2003; Casjens, 2003). Although previously considered genetic baggage, recent evidence suggests that these cryptic phage elements encode functional proteins that have numerous benefits to their hosts (Casjens, 2003; Wang et al., 2010). This extends beyond their ability to contribute to biofilm formation. For example, Wang and colleagues have recently shown that many of the cryptic prophages in BW25113 contribute to traits such as oxidative-, osmotic- and acid-stress resistance, as well as antibiotic resistance. They also found that many of these prophages actually increased growth rate (Wang et al., 2010).

One of these prophage, DLP12, a defective lambdoid prophage present at 12 min of the E. coli genome, has probably been around for more than 100 million years, since it is also found in Salmonella (Casjens, 2003; Lawrence & Ochman, 1998). DLP12 has recently been shown to contribute to stress resistance and biofilm formation in E. coli (Wang et al., 2010). While the exact mechanisms behind the multifaceted effects of DLP12 on host physiology remain unclear, Toba et al. (2011) recently demonstrated that deletion of the lysis genes of this lambdoid-like prophage (the holin S encoded by essD, the lysozyme R, encoded by ybcS, and the spannin Rz/Rz1, encoded by rzpD/rzoD) reduced biofilm formation by affecting curli production in a curli-overproducing K12 derivative. This evidence suggests that specific defective prophage genes are affecting host cell physiology and have been co-opted by the host because of their contributions to host survival.

The lysis cassette of lambda (λ) phage itself encodes a holin, an endolysin and a spannin, which work together to rupture the cell envelope and allow viral particles to escape their cellular confines (Berry et al., 2008; Young et al., 2000). Phage λ regulates expression of these lysis genes through the production of Q, an antiterminator that associates with the RNA polymerase (RNAP) holoenzyme and permits readthrough past an intrinsic terminator located near the lysis cassette promoter P9 (Guo & Roberts, 2004). In the absence of Q, the majority of transcripts produced from P9 fail to extend past the terminator upstream of the lysis genes, thus preventing unnecessary expression of these potentially lethal genes (Oppenheim et al., 2005; Salazar & Asenjo, 2007; Zhou et al., 2006). Q, however, may bind to DNA found adjacent to sigma factor binding regions called the Q binding element (QBE), enabling Q to interact with paused RNAP and become stably incorporated into the elongation complex (Deighan & Hochschild, 2007; Nickels et al., 2002). This event releases RNAP from its stalled state and renders the complex resistant to downstream terminators.

Lindsey et al. (1989) mapped the genome of DLP12 and noted the presence of an ORF upstream of the lysis cassette that encodes a Q-like protein. Sequence alignment between QDLP12 (ybcQ) and Q21 shows 84% sequence identity, strongly suggesting that QDLP12 has a function similar to that of the canonical Q antiterminators, although there is an insertion sequence between Q and the DLP12 lysis genes in K12 (Casjens, 2003; Lindsey et al., 1989).

In this work we present evidence that QDLP12 encodes a functional protein that regulates expression of the DLP12 lysis cassette by binding directly to its promoter and in turn influences biofilm formation in E. coli. Taken together, these data present a demonstration of a Q-like protein regulating expression of functional lysis genes in a defective phage and suggest that a well-characterized regulatory mechanism governing phage lysis cassette expression has been co-opted by the bacterial host.

**METHODS**

**Bacterial strains and growth conditions.** E. coli strain PHL628 is an MG1655 derivative with an ompR234 mutation (Vidal et al., 1998). E. coli PHL628 ΔASDLP12 (628.1), a strain with S (essD) deleted from the chromosome (Toba et al., 2011), was utilized in cell count, fluorometry and chromatim immunoprecipitation (ChiP) assays unless otherwise stated, because Q overexpression is less toxic in this strain (data not shown). E. coli strains were routinely grown in lysogen broth (LB) supplemented with 50 μl kanamycin ml⁻¹ (Kan) at 37 °C overnight with shaking (150 r.p.m.) and subsequently diluted 1:100 into fresh media and cultured at 30 °C for the experiments unless otherwise stated. For early-stationary-phase lysis assays and promoter studies, low-salt LB (5 g NaCl l⁻¹) was used. When required, plates and media were supplemented with ampicillin (Amp 150 μl ml⁻¹) and chloramphenicol (25 μg ml⁻¹) or spectinomycin (100 μg ml⁻¹). For induction, media was supplemented with 1 mM IPTG according to the experimental needs. For growth curves, overnight LB cultures were diluted 1:100 into fresh media. The new cultures were grown at 30 °C for 36 h in 96-well plates. OD₆₀₀ was measured every 30 min in a μQuant spectrophotometer (Bio-Tek).

**Mutants and plasmid construction.** Deletion mutants were constructed by allele replacement using a λ-RED strategy described elsewhere (Datsenko & Wanner, 2000). Briefly, a chloramphenicol (Cm)-resistance-cassette-interrupted version of the gene of interest was created using PCR-mediated ligation (Choi & Schweizer, 2005). In general, each construct consisted of a 5’ and a 3’ 500 bp homology region to the gene of interest linked by a 1 kb Cm cassette flanked by flip recombinase target (FPT) sites. This linear DNA was transformed into wild-type E. coli PHL628 cell expressing the λ-RED recombinase.

The 5’ end and 3’ end homology regions allowed the RED system to replace the wild-type allele with our interrupted version. Clone screening was carried out on plates supplemented with chloramphenicol. After confirmation of recombination via PCR, the Cm

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marker was removed using FLP-recombinase from plasmid pCP20 (Datsenko & Wanner, 2000). All plasmid vectors are temperature sensitive and were cured from the cells by growth at 43 °C. The markerless knockout mutants were confirmed by PCR and sequencing. Complements were constructed using full wild-type versions of the genes cloned into pBBR1MCS (lactose-inducible) (Kovach et al., 1995). For Q overexpression, two vectors were used: pBBR1MCS (medium copy number) and pOFX (low copy number). Q<sup>DLP12</sup> was cloned into pBBRMC1 and labelled pQ1. A C-His<sub>6</sub> tagged version of Q<sup>DLP12</sup> was cloned into pOFX using BamHI and SacI restriction sites and labelled pQ. For promoter reporter assays, the promoterless gfp vector pBA110 was used (Andersen et al., 1998). Plasmid pscgBp-GFP has been described by Toba et al. (2011). Plasmid pessDp-GFP was constructed by cloning the DLP12 lysis gene promoter, essDp, upstream of the gfp gene in pBA110 using KpnI and XhoI restriction sites (Andersen et al., 1998). Wild-type control strains and mutant control strains were constructed by transformation of empty vectors as required per experiment.

**Biofilm architecture.** Biofilms were grown on MatTek Cultureware glass-bottom dishes for imaging. Overnight cultures were inoculated (1 %, v/v) into 3 ml minimal media (MSM) supplemented with 0.2 % Casamino acids (CA). Dishes were incubated at 30 °C for 72 h with shaking on an orbital shaker at 50 r.p.m. The spent medium was exchanged every 24 h with new medium. After 72 h, biofilms were stained with acridine orange and studied under a Leica confocal microscope. To assess biofilm formation and architectural features, a total of eight Z-image stacks (0.25 μm steps) were obtained at ×40 magnification for each mutant and then analysed with COMSTAT (Heydorn et al., 2000).

**Attachment.** Attachment to PVC surface was studied as described previously (Genevaux et al., 1996). Overnight cultures were diluted 1 : 5 to a total volume of 120 μl in 96-well PVC plates and incubated at 30 °C with shaking (50 r.p.m.) for 16 h. After 16 h, turbidity (OD<sub>600</sub>) was measured in each well. Then, 100 μl of a solution of 1 % crystal violet (CV) was added to each well. After 15 min of incubation at room temperature, wells were thoroughly washed with water. Plates were allowed to dry for 15 min at room temperature. After the plate was dry, 100 μl 95 % ethanol was added to each well and incubated for 10 min at room temperature. The absorbance of the ethanol-solubilized CV (OD<sub>590</sub>) was then measured in each well. The original well turbidity was compared with the CV absorbance (OD<sub>590</sub>/OD<sub>600</sub>). Experiments were done in quadruplicate.

**Autoaggregation.** Cell autoaggregation was measured as described previously (Barrios et al., 2006). Briefly, 4 ml overnight (30 °C) LB culture were incubated statically for 12 h at 30 °C. OD<sub>600</sub> was measured from samples taken from the top 5 mm of each culture with minimal disturbance. Then, cultures were vortexed and sampled again. OD<sub>600</sub> values (unvortexed/vortexed) for each mutant were compared as a measurement of cell autoaggregation. Experiments were done in quadruplicate.

**Electron microscopy.** Cells were grown in low-salt LB at 30 °C with shaking overnight. 300 mesh Formvar copper grids (EMS) were floated on 25 μl of the overnight cultures for 1 min. Then the grids were transferred to a solution containing 3 % ammonium molybdate (3 % AMB) pH 7. Grids were then rinsed in MilliQ water for 1 min and allowed to dry before observation. Cells were examined with a Philips Electron Microscope at different magnifications ranging from ×7000 to ×45000. Snap shots were taken using a MicroFire digital camera and software from Optronics at ×10000 magnification.

**Antiterminator predictions.** essDp sequence encompassing 400 bp upstream of the translation start site was analysed for the presence of putative Rho-independent transcriptional terminators using FindTerm software from Softberry (Hagen et al., 2010). The resulting terminator site was mapped by inserting the terminator sequence (58 bp) into the miflD RNA folding program.

**Extracellular β-galactosidase activity assay.** To assess the lysis of the different strains, culture samples were obtained at 36 h and β-galactosidase activity was determined in the cell-free supernatant using ONPG. The production of ortho-nitrophenol (ONP) from ONPG in the supernatant was used as an indicator of the relative abundance of extracellular β-galactosidase. ONP was measured in a Bio-Tek Synergy HT spectrophotometer (OD<sub>405</sub>). Activity was normalized to OD<sub>600</sub> of each respective culture.

**Promoter fusion studies.** Reporter plasmids pscgBp-GFP (Toba et al., 2011) and pessDp-GFP containing the curl and essD promoters, respectively, were transformed into each of the PHL628 strains and selected on low-salt LB Amp plates. Cells were allowed to grow with shaking (150 r.p.m.) in low-salt LB at 30 °C. At indicated time points, fluorescence was measured using a Bio-Tek Synergy HT spectrophotometer with a 485/20 excitation filter and a 530/25 emission filter and normalized to the OD<sub>600</sub> of the corresponding culture. Measurements were done in quadruplicate.

**Quantitative real-time reverse transcription PCR assay.** Cells were cultured overnight with shaking (150 r.p.m.) at 37 °C in LB with 0.2 % glucose and diluted 1 : 100 in low-salt LB with glucose. Diluted cells were grown at 30 °C, for the ΔQ pQ1 strain, glucose was omitted from the culture to allow for Q expression from the lac promoter. At 12 h, cultures were pelleted and lysed. RNA was extracted using a standard phenol/chloroform extraction protocol. Briefly, cell pellets were resuspended (50 mM sodium acetate, 10 mM EDTA pH 5.5) then lysed by addition of 1 % SDS and an equal volume water-saturated phenol (pH 4.3). Lysate was centrifuged for 15 min at 4 °C at 2000 g and poured into a new tube. An equal volume of chloroform was added, then mixed by inverting, centrifuged as before, then the aqueous phase was transferred to a new tube. RNA was isolated from supernatant using 2-propanol precipitation. RNA was treated with DNase I to remove residual DNA contamination. The pure RNA was subsequently used in the RT-PCR assay. A 1 μg

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**Table 1. Primer sequences used in RT and ChiP assays**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>For Tran primer</td>
<td>5’-GATAAAATATTCTACTAATCAATGTTG</td>
</tr>
<tr>
<td>SRRz Rev 685 primer</td>
<td>5’-CACCTCGAGCAACAAGCGGGGTG</td>
</tr>
<tr>
<td>essDp Rev no term. primer</td>
<td>5’-CGGTTTAAAGCTGTGGTACGAGAGTT</td>
</tr>
<tr>
<td>essDp For downstream of term. primer</td>
<td>5’-CGTCTTGTCTGGAGGTTGGGGG</td>
</tr>
<tr>
<td>SRRz Rev primer</td>
<td>5’-CTATCTGACGTCATATAATAC</td>
</tr>
<tr>
<td>5’ BglF For primer</td>
<td>5’-GGAGTATAGCCGAGAAAAATAGTC</td>
</tr>
<tr>
<td>5’ BglF Rev primer</td>
<td>5’-GTACCTCTGTGCTGGCTTTTGT</td>
</tr>
</tbody>
</table>

**Q-DLP12 regulates lysis cassette expression**
sample of purified RNA was incubated with a reverse primer specific to the readthrough essDp transcript (transcription past the putative intrinsic terminator discussed below) in 11 µl total volume at 70 °C for 5 min. Mixtures were chilled on ice for 5 min and then added to a 25 µl reverse transcription reaction comprised of 1 x AMV RT Buffer (Promega), 1 mM dNTP mixture, 40 U rRNasin Inhibitor (Promega) and 10 U AMV reverse transcriptase (Promega). Reactions were incubated for 1 h at 48 °C. Reactions were terminated by incubating for 2 min at 80 °C. One microlitre of cDNA from RT reactions was used as template for PCRs containing a forward essDp transcript primer (Forward Tran Primer, Table 1) which anneals to the beginning of the transcript and the same reverse primer used to generate the cDNA (Table 1). PCR products were run on 1 % agarose gels, stained with ethidium bromide and visualized with a UV transilluminator.

**Purification of QDLP12.** Q-His was cloned into pRSET-A and transformed into BL21DE3 pLysS. Cells were grown at 37 °C with shaking to OD600 0.6. Then expression was induced with 1 mM IPTG followed by a 4 h incubation. Cells were centrifuged, lysed and processed as described in the PrepEase His-Tagged Purification Denaturing Protocol. Purified QDLP12 fractions were analysed by SDS-PAGE, pooled and dialysed against decreasing urea concentrations (4 M–1 M) at 4 °C. The final dialysis at 4 °C was performed into storage buffer [10 mM KH2PO4 pH 6.5, 1 M KCl, 5 mM tris (2-carboxyethyl) phosphine (TCEP), 1 mM EDTA, 50 % (v/v) glycerol]. Protein was stored at -20 °C.

**Electrophoretic gel mobility shift assay of QDLP12 DNA binding.** A 151 bp double-stranded DNA probe encompassing nucleotides +286 to +65 of essDp was made by PCR with a Cy5-labelled primer and then gel purified. QDLP12 protein in storage buffer (10 mM KH2PO4 pH 6.5, 1 M KCl, 5 mM TCEP, 1 mM EDTA, 50 % glycerol) was diluted 1:4 into 16 binding buffer [20 mM Tris/HCl (pH 8.0), 25 mM KCl, 12 % glycerol, 0.1 mM EDTA], prior to mixing with 7.5 ng purified probe and a 100-fold excess of sonicated herring sperm DNA (competitor DNA) in a 25 µl binding reaction; the final buffer was 20 mM Tris/HCl (pH 8.0), 25 mM KCl, 12 % glycerol, 0.1 mM EDTA], prior to mixing with 7.5 ng purified probe and a 100-fold excess of sonicated herring sperm DNA (competitor DNA) in a 25 µl binding reaction; the final buffer was 20 mM Tris/HCl (pH 8.0), 25 mM KCl, 12 % glycerol, 0.1 mM EDTA]. Protein was stored at 4 °C.

**Table 2. ΔQDLP12 mutants exhibit defects in biofilm formation**

<table>
<thead>
<tr>
<th></th>
<th>Biomass (µm³/µm²)</th>
<th>Mean thickness (µm)</th>
<th>Attachment (OD₅₉₀/OD₆₀₀)</th>
<th>Autoaggregation OD₆₀₀ (unvortexed/vortexed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHL 628</td>
<td>6.289 (1.117)</td>
<td>6.544 (0.996)</td>
<td>3.393 (0.479)</td>
<td>0.234 (0.014)</td>
</tr>
<tr>
<td>PHL 628 ΔQ</td>
<td>1.669 (0.722)</td>
<td>1.654 (0.706)</td>
<td>2.298 (0.241)</td>
<td>0.772 (0.044)</td>
</tr>
<tr>
<td>PHL 628 ΔQ pQ1</td>
<td>4.721 (0.838)</td>
<td>4.912 (0.747)</td>
<td>3.384 (0.515)</td>
<td>0.274 (0.054)</td>
</tr>
</tbody>
</table>

Biofilms were grown in MatTek microwell dishes in minimal media with Casamino acids for 72 h at 30 °C with moderate shaking (50 r.p.m.). Biofilm features were determined as described in Methods. The ΔQDLP12 mutant was affected for biomass, thickness, attachment and autoaggregation (α=0.05). These phenotypes were rescued by complementation (PHL 628 ΔQ pQ1). Experiments were done in quadruplicate. Standard error is indicated in parentheses.
Samples were electrophoresed at 4 °C in a Bio-Rad Protean III apparatus in an ice-water bath. Gels were scanned with a Typhoon Imager and fluorescent bands were quantified using ImageJ. The dissociation constant ($K_d$) is reported as the concentration of QDLP12 protein giving 50% shift.

**Chromatin immunoprecipitation assay.** Strain 628.1 pQ was grown with shaking at 30 °C in low-salt LB for 12 h either with or without 1 mM IPTG. Cultures were collected and cross-linked. Cross-linking of protein–protein and protein–DNA complexes was induced by the addition of formaldehyde to a final concentration of 1% for 20 min at 20 °C. The cross-linking reaction was then quenched by the addition of glycine (0.5 M final concentration). Cells were pelleted and washed twice in cold TBS, resuspended in 0.5 ml lysis buffer [10 mM Tris (pH 8), 50 mM NaCl, 10 mM EDTA, 2 mg lysozyme ml$^{-1}$, 1 mM PMSF] and incubated for 30 min at 37 °C. The lysate was then equilibrated in 0.5 ml 2× immunoprecipitation buffer [1× IP buffer: 50 mM HEPES (pH 7), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM PMSF] for 10 min at 4 °C. The DNA was sheared by sonication to an average size of ~500 bp. Following centrifugation (12000 g for 10 min), the lysate was pre-cleared by incubation with 50 µl protein-A CL4B Sepharose slurry (Invitrogen) for 1 h at 4 °C. Samples of the pre-cleared lysates were retained as unenriched input samples. Protein–DNA complexes were immunoprecipitated using monoclonal antibody reactive against the β-subunit of RNAP (Neolone), or a polyclonal antibody reactive against the His tags (Sigma) and 25 µl protein-A CL4B Sepharose. A mock immunoprecipitation control contained only the protein-A Sepharose. Complexes were washed five times with cold IP buffer, once with cold IP buffer containing 0.4 M NaCl and once with cold Tris-EDTA buffer [50 mM Tris (pH 7.5), 10 mM EDTA]. Immunoprecipitated protein/DNA complexes and the input samples were then incubated in elution buffer (TE buffer containing 1% SDS) for 10 min at 65 °C. RNaseA was added (100 µg ml$^{-1}$ final concentration) and incubated for 90 min at 42 °C. The cross-links were reversed by boiling samples for 10 min. The immunoprecipitated DNAs, as well as the products of the mock immunoprecipitation controls and the input samples, were purified using Zippy spin columns. Multiplex PCR was employed to quantify the relative enrichment at exoDp both proximal to (immediately following) the

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**Fig. 2.** (a) ΔQDLP12 mutant exhibits reduced curli phenotype. Electron micrographs were prepared by floating cells on 300 mesh Formvar grids which were washed and then negatively stained with 3% AMB. Grids were visualized with a Philips Electron Microscope 201. Arrows indicate curli fibres. (b) ΔQDLP12 exhibits decreased csgBp expression. Cells bearing the reporter vector pJBA110 carrying the csgB promoter (csgBp) fused to a short-lived GFP were studied for gene expression. Fluorescence was read using a Bio-Tek Synergy HT spectrophotometer with a 485/20 excitation filter and a 530/25 emission filter and normalized to OD$_{600}$. ΔQDLP12 mutant showed reduced curli operon expression, which was restored by complementation with pQ1. Experiments were done in quadruplicate.
transcription start site and within the DLP12 lysis genes. These amplicons were normalized to that of the 5' region of bglF. For primers see Table 1. PCRs were performed with 30 amplification cycles. PCR products were run on 1% agarose gel, stained with ethidium bromide, and bands were quantified using ImageJ software.

**Statistical analyses.** The statistical differences between treatments were determined by calculating the P-values derived from a two-tailed Student’s t test using Microsoft Excel.

**RESULTS**

**Effects of ΔQ<sup>DLPL12</sup> on growth and biofilm formation**

Growth curves performed with wild-type cells, ΔQ<sup>DLPL12</sup>, mutants and ΔQ<sup>DLPL12</sup> mutants complemented with pQ1 (a medium-copy-number plasmid that encodes Q<sup>DLPL12</sup>) showed no significant difference in the time required to reach equivalent cell densities upon entry into stationary phase (data not shown). However, the ΔQ<sup>DLPL12</sup> mutant exhibited significantly attenuated biofilm development (P<0.05) (Fig. 1 and Table 2). The total biofilm biomass and thickness of ΔQ<sup>DLPL12</sup> biofilms decreased fourfold when compared with wild-type biofilms (Table 2). ΔQ<sup>DLPL12</sup> cells also exhibited a 33% reduction in attachment versus wild-type cells (Table 2) and were threefold less capable of aggregating. Exogenous complementation of the ΔQ<sup>DLPL12</sup> mutant with pQ1 restored biofilm formation, attachment and autoaggregation to wild-type levels (Fig. 1 and Table 2).

**Effects of ΔQ<sup>DLPL12</sup> on curli production**

Transmission electron micrographs of the mutant revealed that it had far fewer curli than the wild-type (Fig. 2a). GFP fluorescence from the ΔQ<sup>DLPL12</sup> strain containing the p<sup>csgBp-GFP</sup> was reduced sevenfold compared with the wild-type, indicating that decreased csgB transcription was responsible for the reduced-curli phenotype (Fig. 2b). Expression of Q<sup>DLPL12</sup> from pQ in the ΔQ<sup>DLPL12</sup> strain restored wild-type levels of curli expression (Fig. 2a, b).

**Intrinsic transcriptional terminator prediction**

Since lambdoid Q proteins function as antiterminators, we postulated that Q<sup>DLPL12</sup> would be acting in a similar manner. We queried the promoter region using Softberry and found a potential terminator ~100 bp upstream of the translation start site. We next mapped the structure of the putative terminator by inserting the essDp sequence from 2138 bp to 280 bp relative to the translation start site using mfold (Fig. S1 available with the online version of 0500 1000 1500 2000 2500 3000 3500 4000 4500 5000 628.1 pDp-GFP 628.1 pQ 628.1 pQ (a) PessD-GFP expression (fluorescence/OD600) (b) ΔQ PHL628 essD16S ΔQ essD16S pQ (-) ΔQ pQ1 (+) +IPTG ΔQ pQ1 (-) -IPTG PHL628 ΔQ essD16S (ΔQ pQ1 (-) -IPTG) ΔQ pQ1 (+) +IPTG Fig. 3. Q<sup>DLPL12</sup> upregulates transcription of essDp. (a) Fluorescence from pessDp-GFP was quantified to assess the effects of Q expression on essDp transcription 12 h after IPTG addition. Overnight cultures were diluted in fresh LB and allowed to grow at 30 °C in the presence (black bars) or absence (white bars) of 1 mM IPTG. Fluorescence was read using a Bio-Tek Synergy HT spectrophotometer with a 485/20 excitation filter and a 530/25 emission filter and normalized to OD<sub>600</sub>. (b) The wild-type strain and the ΔQ<sup>DLPL12</sup> mutant were grown in low-salt LB. At 12 h RNA was harvested from cells, quantified and subjected to RT-PCR. The amount of readthrough transcript (essD) was normalized to that of 16S rRNA transcript. The ΔQ<sup>DLPL12</sup> mutant exhibited significantly reduced levels of readthrough transcript when compared with the wild-type. IPTG-mediated overexpression of Q<sup>DLPL12</sup> [indicated by (+)] increased the relative abundance of essD mRNA. Experiments were quantified in triplicate (one representative result shown in the above images).**
this paper). The structure consisted of a hairpin and had a $\Delta G$ value of $-12.5$ kcal mol$^{-1}$. This suggests that $essDp$ contains an intrinsic transcriptional terminator.

**Q<sup>DLPI2</sup> regulates transcription of the DLP12 lysis cassette**

The prediction of an intrinsic terminator suggested to us that $Q<sup>DLPI2</sup>$ regulated the DLP12 lysis genes in a manner analogous to that of other lambdoid Q proteins. To test this hypothesis, $Q<sup>DLPI2</sup>$ was overexpressed in wild-type cells and cell lysis was inferred by the release of $\beta$-galactosidase activity into the supernatant. $Q<sup>DLPI2</sup>$ overexpression promoted a 27-fold increase in $\beta$-galactosidase activity in the supernatant, implying that it was causing cell lysis (data not shown). Consistent with that observation, we found that IPTG-induced overexpression of $Q<sup>DLPI2</sup>$ caused a 22-fold increase in GFP fluorescence from the $essDp$-GFP promoter reporter (Fig. 3a). These results were of similar magnitude to those obtained using semiquantitative RT-PCR which provided evidence for a sevenfold increase in the relative abundance of the read-through $essDp$ transcript when $Q<sup>DLPI2</sup>$ was overexpressed (Fig. 3b). Deletion of $Q<sup>DLPI2</sup>$ resulted in a modest 60% decrease in the basal production of read-through transcript from $essDp$ when compared with the wild-type (Fig. 3b). These results were all highly significant ($P<0.05$) and support the hypothesis that $Q<sup>DLPI2</sup>$ functions as a positive regulator of the lysis cassette.

**Q<sup>DLPI2</sup> overexpression results in RNA polymerase enrichment at $essDp$ in vivo**

We sought to determine whether $Q<sup>DLPI2</sup>$ increased the amount of RNAP at the lysis cassette promoter in vivo. We
This study shows that Q^{DLP12} positively regulates the overexpression of the DLP12 lysis genes, and, in turn, regulates late gene expression through direct binding to the QBE DNA site so we attempted to determine whether Q^{DLP12} directly bound the promoter of the DLP12 lysis cassette. We purified Q^{DLP12} and employed a gel mobility shift assay to assess its ability to bind essDp. Fig. 5 shows that there was an observable band shift, indicating that Q^{DLP12} binds the lysis cassette promoter. The presence of 100-fold excess non-specific competitor DNA failed to block Q from causing a shift, whereas a 10-fold excess of unlabelled probe prevented most of the labelled probe from shifting up. These results confirm that Q’s interaction was specific to essDp (K_d 5.3 μM) (Fig. 5).

**DISCUSSION**

In lambda, Q^7 regulates late gene expression through direct binding to the QBE DNA site so we attempted to determine whether Q^{DLP12} directly bound the promoter of the DLP12 lysis cassette. We purified Q^{DLP12} and performed chromatin immunoprecipitation (ChIP) on cells expressing Q-His^{DLP12} using anti-His and anti-RNAP beta subunit (antiβ) antibodies. Upon Q-His^{DLP12} overexpression, analysis of the antiβ pulldown revealed that there was a significant increase in the relative abundance of RNAP (29-fold) at the region of essDp immediately downstream of the transcription start site (promoter-proximal site) when compared with the control (Fig. 4). There was also a marked increase in RNAP enrichment downstream of the intrinsic terminator as a result of Q^{DLP12} overexpression (fivefold), which corroborates the RT-PCR results showing increased abundance of readthrough transcript (Figs 3 and 4). Repeated attempts to pull down Q-His^{DLP12} with either anti-His antibodies or with nickel columns were unsuccessful.

**Q^{DLP12} binds to essDp DNA in vitro**

In lambda, Q^7 regulates late gene expression through direct binding to the QBE DNA site so we attempted to determine whether Q^{DLP12} directly bound the promoter of the DLP12 lysis cassette. We purified Q^{DLP12} and employed a gel mobility shift assay to assess its ability to bind essDp. Fig. 5 shows that there was an observable band shift, indicating that Q^{DLP12} binds the lysis cassette promoter. The presence of 100-fold excess non-specific competitor DNA failed to block Q from causing a shift, whereas a 10-fold excess of unlabelled probe prevented most of the labelled probe from shifting up. These results confirm that Q’s interaction was specific to essDp (K_d 5.3 μM) (Fig. 5).

**DISCUSSION**

This study shows that Q^{DLP12} positively regulates the expression of the DLP12 lysis genes, and, in turn, influences biofilm formation in *E. coli*. Our work implicates Q^{DLP12} in direct expression of the DLP12 lysis genes, where it probably acts as an antiterminator like other Q homologues (Guo & Roberts, 2004). The fact that ΔQ^{DLP12} mutants behave almost identically to the lysis cassette knockouts (Toba et al., 2011) with respect to biofilm defects (Figs 1 and 2 and Table 2) suggests that the two cistrons are involved in the same physiological pathway. Curli production was rescued in the lysis cassette mutants by deleting nagK (Toba et al., 2011), demonstrating that curli regulation was sensitive to changes in peptidoglycan recycling. Thus, we expected that loss of Q^{DLP12} would affect curli production given our evidence that Q^{DLP12} regulates the lysis cassette. Future work, however, needs to be done to elucidate the exact manner in which Q^{DLP12} affects curli expression.

Prophage genes have been shown to affect bacterial physiology in many different ways including altering growth rate (Wang et al., 2010), escaping host immune responses (*bor* and *lom*) (Barondess & Beckwith, 1995; Vica Pacheco et al., 1997), altering endogenous mutation rates (Chikova & Schaaper, 2006; Pal et al., 2007), increasing resistance to antibiotics and biocides (Wang et al., 2010) and altering biofilm formation (Rice et al., 2009; Wang et al., 2010; Webb et al., 2004). In some cases the effect on biofilm formation was a direct result of the production of filamentous prophage such as Pf1 and Pf4 in *Pseudomonas aeruginosa* (Rice et al., 2009; Webb et al., 2004). In other cases the effect was more subtle and not well understood from a mechanistic standpoint (Wang et al., 2010). This is especially true for defective prophages, which have long been considered genetic baggage (Casjens, 2003), since they have lost the ability to excise and replicate.

Recent evidence has shown that some defective prophages can still influence the physiology of their bacterial hosts...
(Toba et al., 2011; Wang et al., 2010). There are no published reports, however, demonstrating a role for defective-prophage-encoded antiterminators contributing to the regulation of host cell processes such as curli production. Transcriptional antitermination occurs within bacterial operons such as hly, rfa and kps which are involved in the production of haemolysin, lipopolysaccharide and exopolysaccharide, respectively (Bailey et al., 1997; Santangelo & Roberts, 2002). Expression of these genes is dependent upon the host-encoded antiterminator, rfaH, which interacts with the RNAP holoenzyme in a manner analogous to that of Q (Bailey et al., 1997; Santangelo & Roberts, 2002). While we have provided in vivo and in vitro evidence demonstrating that QDLP12 directly regulates lysis cassette expression, the exact mechanism requires further elucidation. In addition, there is a need for more information about the signals that regulate expression of QDLP12 itself. A putative sigma-32-specific promoter has been predicted 182 bp upstream of the translation start site of QDLP12 (Huerta & Collado-Vides, 2003), although neither heat (50 °C) nor ethanol exposure were sufficient to upregulate QDLP12 transcription in our hands (data not shown).

Taken together, this work demonstrates that overexpression of QDLP12 upregulates expression of the DLP12 lysis cassette due to the direct interaction of QDLP12 with essDp. To our knowledge, this is the first report of a functional Q from a defective prophage that is relevant to normal host physiology and suggests that this well-characterized phase mechanism of transcription regulation has been co-opted by E. coli in a novel way.

ACKNOWLEDGEMENTS

This research was supported in part by a State University of New York (SUNY) Minority Fellowship and a Cornell Provost’s Diversity Fellowship to K.G.R. and in part by grants from the United States Department of Agriculture (USDA). We are grateful to Jeff Roberts and Jeremy Bird for helpful discussions and insights regarding Q purification and EMSAs.

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Edited by: R. Palmer