A transcriptome study of the QseEF two-component system and the QseG membrane protein in enterohaemorrhagic *Escherichia coli* O157:H7

Nicola C. Reading,¹ David Rasko,^{1†} Alfredo G. Torres² and Vanessa Sperandio^{1,3}

Correspondence
Vanessa Sperandio
vanessa.sperandio@
utsouthwestern.edu

¹Department of Microbiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-9048, USA

²Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1070, USA

³Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-9048, USA

QseE is a sensor kinase that responds to epinephrine, sulfate and phosphate. QseE constitutes a two-component signalling system together with the QseF σ^{54} -dependent response regulator. Encoded within the same operon as *qseEF* is the *qseG* gene, which encodes a membrane protein involved in the translocation of a type III secretion effector protein of enterohaemorrhagic *Escherichia coli* (EHEC) into epithelial cells. The *qseEGF* genes also form an operon with the *glnB* gene, which encodes the *E. coli* nitrogen sensor PII protein. Here we report a transcriptome analysis comparing *qseE*, *qseF* and *qseG* single mutants with the wild-type strain. This study revealed that the proteins encoded by these genes play a modest but significant role in iron uptake. Although QseEFG regulate genes involved in nitrogen utilization, these proteins do not play a notable role in nitrogen metabolism. In addition, QseEFG regulate transcription of the *rscBC* and *phoPQ* two-component systems, linking several signal transduction pathways. The similarity of the microarray profiles of these mutants also indicates that these proteins work together. These data indicate that QseEFG are involved in the regulation of virulence and metabolism in EHEC.

Received 23 July 2009
Revised 15 December 2009
Accepted 4 January 2010

INTRODUCTION

With environmental signalling playing such an important role in the adaptation by bacteria to different niches, the importance of a system to recognize and transduce signals that indicate the external or internal environment is paramount. Histidine sensor kinases are the major environmental sensory proteins in bacteria, and bacterial genomes encode many of these proteins. Histidine sensor kinases usually act in concert with a response regulator protein, constituting a two-component system. The classical two-component systems in Gram-negative bacteria are

composed of an integral inner membrane histidine sensor kinase protein and a cytosolic response regulator protein. The periplasmic region of the sensor kinase is able to sense external signals and catalyse an autophosphorylation reaction. Once phosphorylated, the sensor kinase is able to transfer the phosphoryl group to a conserved aspartate residue on the cytosolic response regulator. This enables the regulator to act on downstream genes, most often activating transcription (Hoch, 2000).

As well as adapting to environmental niches, many two-component systems are intimately involved in virulence. In *Streptococcus pneumoniae*, eight of the 13 known systems are involved in virulence in a mouse model (Throup *et al.*, 2000). Other examples of the roles of two-component systems in virulence are: persistence in infection by *Mycobacterium tuberculosis* (Zahrt & Deretic, 2001), invasion and antimicrobial resistance in *Salmonella typhimurium* (Bader *et al.*, 2005; Smith *et al.*, 2008), and adhesion of *Escherichia coli* (Otto & Silhavy, 2002). In the *E. coli* K-12 genome there are 29 histidine kinases and 32

[†]Present address: Department of Microbiology and Immunology, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

Abbreviations: AE, attaching and effacing; DPD, 2,2'-dipyridyl; EHEC, enterohaemorrhagic *Escherichia coli*; LEE, locus of enterocyte effacement.

The microarray data discussed in this paper are available from Gene Expression Omnibus (GEO) via accession number GSE12831.

response regulators (Mizuno, 1997). The quorum-sensing *E. coli* regulators E, F and G contain the components of a traditional two-component system. These proteins are conserved among numerous enteric organisms, including species of *Shigella*, *Salmonella*, *Yersinia* and *Klebsiella*, and *E. coli*. The genes encoding these proteins are co-transcribed in an operon with *glnB*. QseE (GrlK) is a sensor kinase that responds to the host hormone epinephrine, phosphate and sulfate. It contains two transmembrane domains, a signal histidine kinase domain and an ATPase domain (Reading *et al.*, 2007, 2009; Reichenbach *et al.*, 2009). QseF (GrlR) is a two-component response regulator protein containing a response regulator domain with a key aspartate residue, a σ^{54} -interaction domain including the GAFTGA motif, and a helix-turn-helix DNA-binding domain. QseG is an outer membrane protein and shares homology with many α -helical proteins (Reading *et al.*, 2007, 2009; Reichenbach *et al.*, 2009). The *glnB* gene encodes the PII protein. PII is a signal transduction protein involved in the modulation of the kinase and phosphatase activities of the nitrogen sensor kinase NtrB in *E. coli*. Upon autophosphorylation, NtrB phosphorylates the NtrC σ^{54} -dependent response regulator, which then binds to DNA to activate transcription of genes encoding metabolic enzymes and permeases in response to carbon and nitrogen status in *E. coli* (Ninfa & Atkinson, 2000; Ninfa *et al.*, 2000). The kinase and phosphatase activities of NtrB are regulated by PII, which upon binding to NtrB inhibits the kinase activity and activates the phosphatase activity (Ninfa & Atkinson, 2000; Ninfa *et al.*, 2000).

In enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7, QseE, F and G are involved in virulence. EHEC is an enteric pathogen that causes disease worldwide, leading to cases of haemorrhagic colitis and life-threatening haemolytic uraemic syndrome (Kaper *et al.*, 2004). EHEC colonizes the large intestine and is able to tightly adhere to intestinal epithelial cells. Among the virulence factors of EHEC is the ability to form attaching and effacing (AE) lesions on intestinal epithelial cells, which efface the microvilli and reorganize the host cell cytoskeleton into a pedestal-like structure (Kaper *et al.*, 2004). Most of the genes necessary for EHEC to form AE lesions are encoded in a region known as the locus of enterocyte effacement (LEE) (McDaniel *et al.*, 1995). This region is composed of 41 genes, and encodes all the components of a type III secretion system, an outer membrane adhesin, intimin, and an effector named translocated intimin receptor (Tir) (Elliott *et al.*, 1998; Jarvis *et al.*, 1995; Jerse *et al.*, 1990; Kenny *et al.*, 1997). Tir is translocated through the type III secretion machinery into eukaryotic host cells. This protein embeds itself in the eukaryotic membrane and acts as a receptor for intimin. In addition, Tir initiates a signalling cascade that leads to actin nucleation and the formation of the AE lesion (Kenny *et al.*, 1997). EHEC also harbours numerous type III-secreted effectors encoded outside the LEE region that are also translocated via the LEE-encoded type III secretion system (Deng *et al.*, 2004; Tobe *et al.*,

2006). One such effector molecule is EspFu (TccP), which acts with Tir to promote AE lesion formation (Campellone *et al.*, 2004; Cheng *et al.*, 2008; Garmendia *et al.*, 2004).

QseE autophosphorylates and then transfers a phosphate to QseF (Yamamoto *et al.*, 2005), which in turn activates transcription of *espFu/tccP* in EHEC (Reading *et al.*, 2007). The QseG outer membrane protein is involved in Tir translocation by the type III secretion system (Reading *et al.*, 2009). The concerted action of these three proteins facilitates AE lesion by EHEC on host epithelial cells. The aim of this study was to elucidate the global role of the QseEFG, by examining the transcriptional profiles of cells with non-polar mutations in *qseE*, *qseF* or *qseG*, compared with the wild-type (WT). Here we show that QseEFG also regulate metabolism, iron uptake and other two-component systems, suggesting other roles for this three-component system beyond AE lesion formation in EHEC.

METHODS

Strains and plasmids. All bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* strains were grown in aerobic conditions in low-glucose Dulbecco's modified Eagle's medium (DMEM) at 37 °C for microarray studies and real-time PCR. All overnight cultures were grown in Luria-Bertani (LB) broth at 37 °C unless otherwise noted. Antibiotics for culture growth were added at the following concentrations: ampicillin, 100 µg ml⁻¹; chloramphenicol, 30 µg ml⁻¹; kanamycin, 50 µg ml⁻¹; and tetracycline, 25 µg ml⁻¹.

Recombinant DNA techniques. PCR, restriction digestions, plasmid preparations, transformations, gel electrophoresis and ligations were all performed according to standard methods (Sambrook *et al.*, 1989). Primers used in real-time PCR are listed in Table 2.

RNA extraction. Cultures of 86-24, NR01, NR02 and NR03 were grown aerobically in LB medium at 37 °C overnight and then were diluted 1:100 in low-glucose DMEM and allowed to grow at 37 °C until they reached OD₆₀₀ 1.0. RNA was extracted from three replicates of each strain using a RiboPure bacterial RNA isolation kit (Ambion) according to the manufacturer's instructions.

Microarrays. The GeneChip *E. coli* Genome 2.0 array of the Affymetrix system contains 10 000 probe sets directed towards genes from four different strains of *E. coli*: K-12 laboratory strain MG1655, uropathogenic strain CFT073, O157:H7 enterohaemorrhagic strain EDL933, and O157:H7 enterohaemorrhagic strain Sakai (<http://www.affymetrix.com>). These GeneChips were used in order to compare the transcriptome of 86-24 with strains NR01, NR02 and NR03. Processing of extracted RNA, cDNA labelling, hybridization and slide-scanning procedures were performed according to manufacturer's instructions found in the 'Affymetrix Gene Expression Technical Manual' (<http://www.affymetrix.com>).

Microarray data analysis. The data from scanning a single replicate of the Affymetrix GeneChip *E. coli* Genome 2.0 array for each strain were gathered using GCOS v1.4 as per the manufacturer's instructions. Normalization of data was conducted using Robust Multiarray analysis (Bolstad *et al.*, 2003; Irizarry *et al.*, 2003) at the RMAExpress website (<http://www.rmaexpress.bmbolstad.com>). Output data were analysed for differences in gene expression resulting from the removal of *qseE*, *qseF* or *qseG*.

Table 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Reference or source
Strains		
86-24	Stx2 + EHEC strain (serotype O157:H7)	Griffin <i>et al.</i> (1988)
NR01	86-24 <i>qseE</i> non-polar mutant	Reading <i>et al.</i> (2007)
NR02	86-24 <i>qseF</i> non-polar mutant	Reading <i>et al.</i> (2007)
NR03	86-24 <i>qseG</i> non-polar mutant	Reading <i>et al.</i> (2009)
NR04	NR01 complemented with plasmid pNR01	Reading <i>et al.</i> (2007)
NR05	NR03 complemented with plasmid pNR03	Reading <i>et al.</i> (2009)
NR06	NR02 complemented with plasmid pNR02	Reading <i>et al.</i> (2007)
Plasmids		
pBadMycHis	C-terminal Myc-His-tag cloning vector	Invitrogen
pNR01	<i>qseE</i> in pBadMycHis	Reading <i>et al.</i> (2007)
pNR02	<i>qseF</i> in pBadMycHis	Reading <i>et al.</i> (2007)
pNR03	<i>qseG</i> in pBadMycHis	(38)

Real-time RT-PCR. Primers used in real-time RT-PCR analysis were designed using Primer Express v1.5 (Applied Biosystems) and are listed in Table 2. Real-time RT-PCR analysis was conducted using an Applied Biosystems ABI 7500 sequence detection system using a one-step reaction. Each primer set was checked for amplification efficiency by standard curves resulting from using varying concentrations of RNA template. To ensure template specificity, products were heated to 95 °C for 15 s, cooled to 60 °C, and heated to 95 °C while fluorescence was monitored. To analyse gene expression in NR01, NR02 and NR03 compared with 86-24 *E. coli*, relative quantification analysis was used. Parameters for cDNA generation and amplification were as follows: 48 °C for 30 min, 95 °C for 10 min, and 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The RNA polymerase subunit Z, *rpoZ*, was used as an endogenous control. In each reaction of 20 µl, 10 µl 2 × SYBR master mix, 0.1 µl Multiscribe reverse transcriptase (Applied Biosystems) and 0.1 µl RNase inhibitor (Applied Biosystems) were added.

Detection, quantification and statistical analysis. Applied Biosystems ABI Sequence Detection 1.3 software was used for initial collection of data. Values were normalized to *rpoZ* and analysed using the comparative critical threshold (C_t) value as previously described

(Anonymous, 1997). Expression is shown in graphs as *n*-fold change in expression level compared with WT levels at late exponential growth. Error bars represent the standard deviations of the $\Delta\Delta C_t$ value (Anonymous, 1997). Student's *t* test was performed to assess statistical significance. A *P* value of <0.05 was considered significant.

RESULTS

Microarray analysis of $\Delta qseE$, $\Delta qseF$ and $\Delta qseG$ compared with WT EHEC

We have previously shown that QseEFG in EHEC is involved in AE lesion formation (Reading *et al.*, 2007, 2009). To identify additional targets of QseEFG, we performed transcriptome studies utilizing the Affymetrix GeneChip *E. coli* Genome 2.0 arrays to compare the transcripts of WT EHEC with those of $\Delta qseE$, $\Delta qseF$ and $\Delta qseG$ single mutants. The GeneChip *E. coli* Genome 2.0 Array includes approximately 10 000 probe sets for all 20 366

Table 2. Oligonucleotides used in this study

Primer	Sequence	Description
RcsCF	GCA GGA GAT GGC ACA AGC A	Real-time analysis
RcsCR	TGA CGG TGG CAA GGA ACA T	Real-time analysis
RcsBF	TCT CTC GCC AAA AGA GAG TGA AG	Real-time analysis
RcsBR	CGA TCT CGG TCA CCA GGA A	Real-time analysis
RpoZ RTF	TGC AGG TAG GCG GAA AGG	Real-time analysis
RpoZ RTR	GCG CAG CGC GAT TAC AGT	Real-time analysis
PhoP RTF	CCG CTG GCG TAG CAA TG	Real-time analysis
PhoP RTR	AGC TTT CAC GGG CGG TTA A	Real-time analysis
PhoQ RTF	GCC GCC TGG TGG AGT TTA C	Real-time analysis
PhoQ RTR	TCT TCC AGT TCG CGG ACT TC	Real-time analysis
FepE RTF	GGC GAT TAT TGT GAT CCT TTC C	Real-time analysis
FepE RTR	CGC AAT AAC ACG CTA CCA CAAG	Real-time analysis
EntB RTF	GAT GCG CTG GCC GAT TT	Real-time analysis
EntB RTR	AAC GTC CGG CCA CAT ATT TC	Real-time analysis
TonB RTF	CCC CTG GCC GAC GTT ACT	Real-time analysis
TonB RTR	AGA GCA GAC CCG CCA CAA C	Real-time analysis

genes and several intergenic regions present in four strains of *E. coli*: K-12 (MG1655 laboratory strain), CFT073 (uropathogenic strain), O157:H7 EDL933 and Sakai (enterohaemorrhagic strains) (<http://www.affymetrix.com>). The results of these analyses revealed that across the three mutant strains, 445 probe sets showed increased expression and 130 probe sets showed decreased expression (Fig. 1), while different sets of probes were differentially expressed in each of the mutants. A total of 3057 probe sets were differentially expressed in the *qseE* mutant as compared with the WT strain (Fig. 1), with 1726 showing increased, and 1331 showing decreased expression. Comparisons between the WT and the *qseF* mutants showed that 1405 probe sets were differentially expressed between these strains, and a greater proportion of these genes showed increased expression than decreased expression (1101 increased versus 304 decreased). A similar scenario can be observed comparing the transcriptomes of the WT and the *qseG* mutant, where 1661 probe sets were differentially expressed, with 1092 being increased and 569 being decreased (Fig. 1). The majority of the genes with an altered profile were derived from the *E. coli* MG1655 strain. These features represent a common *E. coli* backbone conserved among all *E. coli* pathovars, and many of the features are associated with central metabolism and core biological processes (Table 3). More similarity was seen in the effect of knocking out these genes, when $\Delta qseE$ and $\Delta qseG$ were compared; however, there was a high degree of overlap between all three mutants, as shown in the Venn diagram in Fig. 1.

Several notable areas of regulation were identified through this screen, including many metabolic genes and numerous outer membrane proteins and transport systems. Of particular interest, genes involved in both iron and nitrogen utilization were regulated by this three-component system (Figs 2 and 3).

QseEFG does not regulate nitrogen metabolism

Given the abundance of metabolic genes that appeared to be differentially regulated in the array, we would expect to

see a difference in growth ability between the WT and each of the mutants. To follow up the microarray results that showed that the genes involved in nitrogen regulation (Fig. 2a) were differentially regulated in the $\Delta qseE$, $\Delta qseF$ and $\Delta qseG$ mutants, we conducted a series of growth experiments. Because *glnB* is in the same operon as *qseEGF*, it is not surprising that nitrogen metabolism gene regulation was altered in the mutant strains. The *glnB* gene encodes the α -ketoglutarate sensor PII. This protein negatively regulates the two-component system NtrB/NtrC, which activates glutamine synthetase when the cell is growing in low-nitrogen media (Ninfa & Jiang, 2005). However, we found no defect in nitrogen utilization in any of the mutants compared with the WT, given that all strains had similar growth rates in minimal media with or without glutamine supplementation (Fig. 2).

We then tested the ability of each mutant to grow on plates containing arginine as the sole nitrogen source. Again, each one of the mutant strains was able to grow on this minimal medium at a rate comparable to that of the WT (data not shown). These data indicate that although this three-component system modulates expression of genes involved in nitrogen metabolism, the $\Delta qseE$, $\Delta qseF$ and $\Delta qseG$ mutants are not defective in nitrogen utilization. This suggests that QseEFG does not overcome the primary nitrogen signalling system, NtrB and NtrC.

QseEFG has a mild effect on iron utilization

The gene array studies suggested that QseEFG modulate expression of several iron-uptake systems. To confirm the array studies, we performed real-time quantitative RT-PCR. Our real-time experiments confirmed that transcription of *entB* was decreased in the *qseG* mutant, while it was unaltered in the *qseE* and *qseF* mutants. Expression of *fepE* was decreased in each of the mutants, while expression of *tonB* was unaltered (Fig. 3a). EntB is involved in the synthesis of the enterobactin siderophore (Hantash & Earhart, 2000), while FepE is involved in enterobactin

Fig. 1. Venn diagrams showing the number of overlapping up- or downregulated genes between the *qseE*, *F* and *G* mutant strains compared with the WT.

Table 3. Pathovar-specific distribution of genes

The total number of genes assigned to the specific genomes included is 10 013. There are an additional 96 features that are used as controls and 99 features that are associated with phages and plasmids and thus not directly linked to a genome project. The total number of features on the array is 10 208.

Comparison	MG1655 (<i>n</i> =4070)		EDL933 (<i>n</i> =1787)		Sakai (<i>n</i> =373)		CFT073 (<i>n</i> =2486)		Intergenic (<i>n</i> =1297)	
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
86-24 versus $\Delta qseE$										
Decrease	871	21.40	263	14.72	33	8.85	127	5.11	33	2.54
Marginal decrease	253	6.22	93	5.20	18	4.83	53	2.13	10	0.77
Increase	558	13.71	406	22.72	83	22.25	235	9.45	395	30.45
Marginal increase	74	1.82	48	2.69	5	1.34	17	0.68	28	2.16
No change	2314	56.85	977	54.67	234	62.73	2054	82.62	831	64.07
86-24 versus $\Delta qseF$										
Decrease	183	4.50	69	3.86	9	2.41	33	1.33	10	0.77
Marginal decrease	280	6.88	114	6.38	24	6.43	37	1.49	14	1.08
Increase	304	7.47	207	11.58	50	13.40	213	8.57	285	21.97
Marginal increase	272	6.68	134	7.50	15	4.02	44	1.77	65	5.01
No change	3031	74.47	1263	70.68	275	73.73	2159	86.85	923	71.16
86-24 versus $\Delta qseG$										
Decrease	332	8.16	158	8.84	23	6.17	38	1.53	14	1.08
Marginal decrease	328	8.06	140	7.83	25	6.70	27	1.09	17	1.31
Increase	300	7.37	250	13.99	49	13.14	176	7.08	287	22.13
Marginal increase	217	5.33	88	4.92	15	4.02	47	1.89	62	4.78
No change	2893	71.08	1151	64.41	261	69.97	2198	88.42	917	70.70

synthesis and transport (Ozenberger *et al.*, 1987) and has been implicated in modification of LPS in *Salmonella* (Murray *et al.*, 2003). TonB confers energy for several iron-transport systems.

To investigate differential iron uptake and utilization in each of the mutant strains, we performed several iron-utilization and uptake assays. As shown in Fig. 3(b), when the mutants were grown on LB plates containing either the iron chelator 2,2'-dipyridyl (DPD) or DPD and haemin (for recovery) for 48 h, there was no difference in the effect of these compounds on any of the mutants compared with the WT. The growth of all strains was inhibited by the addition of 350 μ M DPD and all strains were able to recover to an equal extent with the addition of 8 μ M haemin. When the strains were grown in minimal media (Fig. 3c), no difference in growth was seen. When the strains were grown in minimal media containing DPD, the *qseE* and *qseG* mutants grew slightly more slowly in the presence of 350 μ M DPD (Fig. 3d). This slight defect in growth was complemented when a copy of any one of the genes was expressed in the mutant strains (Fig. 3d). These data indicate that while numerous iron-uptake and -utilization genes are differentially regulated in the mutants, the effect on iron utilization or growth is minimal. However, there did seem to be a small defect in mutant growth under iron-limiting conditions in minimal media, which were the media utilized in the transcriptional studies.

Cross-talk between QseEFG and other two-component signalling systems

Cross-talk has been shown to exist between two-component systems at the protein level by non-cognate phosphorylation (Yamamoto *et al.*, 2005). The gene array studies indicated that numerous two-component signalling systems are regulated by QseEFG at the transcriptional level. To validate these findings, we performed real-time RT-PCR and found that *phoP* expression was downregulated in all three mutant strains, while *phoQ* expression was downregulated in both the *qseF* and the *qseG* mutants (Fig. 4). PhoPQ is a two-component system known to be involved in the sensing of antimicrobial peptides (Bader *et al.*, 2005). In addition, the RcsBC system, which has been shown to modulate expression of the *LEE* region (Tobe *et al.*, 2005), was regulated by QseEFG. Expression of *rcsB* was upregulated in *qseE*, *qseF* and *qseG* mutants, while *rcsC* was only upregulated in the *qseG* mutant (Fig. 4). These data indicate that QseEFG transcriptionally regulate other two-component systems, and provide a link between QseEFG regulation of *espFu* expression, Tir translocation and expression of the *LEE* region.

DISCUSSION

Two-component signalling systems are involved in the recognition and transduction of environmental signals by bacteria, so they can efficiently and effectively adapt to

Fig. 2. *qseEFGglnB* affects nitrogen gene regulation. (a) Heat map showing genes involved in nitrogen regulation. These genes are primarily downregulated in *qseE*, *qseG* and *qseF*. (b) Growth curves showing the growth of each mutant in minimal media and media into which glutamine was titrated. In both cases, there was no significant difference in growth between the WT EHEC and the *qseE*, *qseG* and *qseF* mutant strains.

certain niches. These systems have been shown to be important in bacterial pathogenesis in both Gram-positive and Gram-negative organisms (Laub & Goulian, 2007). In this study, we report a global transcription analysis of the recently described QseEF two-component system and the QseG membrane protein. Through microarray analysis, it was confirmed that many genes are equally affected transcriptionally compared with the WT by knocking out *qseE*, *qseF* and *qseG* (Fig. 1, Table 3). This trend was observed in several sets of genes, including many metabolism

genes. However, within these datasets, independent regulation of some genes by each of the three proteins was also seen.

The genes encoding QseEFG constitute an operon with *glnB*. The *glnB* gene encodes the PII protein, involved in the modulation of the kinase and phosphatase activities of the *E. coli* nitrogen sensor kinase NtrB (Ninfa & Atkinson, 2000; Ninfa *et al.*, 2000). This genetic organization combined with the microarray studies suggested that several nitrogen-utilization genes were regulated by QseEFG and led us to investigate whether *qseE*, *qseF* or *qseG* mutants had any defects in nitrogen utilization. As shown in Fig. 2, several nitrogen-utilization tests suggested that mutants for these genes are indistinguishable from the WT. Altogether, these data suggest that QseEFG play a minor role in regulation of nitrogen-utilization genes, which does not translate into defects in nitrogen utilization, a metabolic phenotype primarily regulated by the NtrBC system.

Many iron-utilization genes were also shown to be under QseEFG regulation (Fig. 3). However, we only observed a mild defect in iron utilization when the strains were grown in DMEM, which is the condition in which the RNA was extracted for microarray analyses. These data suggest that although this system regulates iron uptake in EHEC, the presence of additional iron-utilization mechanisms can overcome the defect exerted by downregulation of QseEFG-controlled iron transport systems.

The *E. coli* genome encodes approximately 32 two-component systems. Although cross-phosphorylation and signalling among non-cognate sensors and response regulators is known to occur, a study by Yamamoto *et al.* (2005) has shown that phosphorylation of non-cognate response regulators by histidine kinases is rare and occurs in only 22 of 692 combinations in *E. coli* (Yamamoto *et al.*, 2005). This same study also showed that some two-component systems are more prone to non-cognate signalling than others; among these systems is QseEFG. This point is illustrated by the fact that QseF is phosphorylated by at least four non-cognate histidine kinases in addition to QseE (Yamamoto *et al.*, 2005). QseEFG affect the regulation of other two-component systems, indicating that QseEFG also communicate with other two-component regulatory systems at the transcriptional level. Indeed, transcription of PhoPQ, a two-component system that senses antimicrobial peptides and magnesium (Bader *et al.*, 2005; Groisman & Mouslim, 2006), is activated by QseEFG. Conversely, transcription of *rcsBC*, a two-component system that regulates flagella (Clemmer & Rather, 2007), and the LEE genes (Tobe *et al.*, 2005) is repressed by QseEFG. RcsBC play a modulatory role in the expression of the LEE genes (Tobe *et al.*, 2005), while QseEFG are involved in activation of *espFu/tccP* (Reading *et al.*, 2007), transcription, and translocation of type III secretion effectors to host cells (Reading *et al.*, 2009). QseEFG regulation of the RcsBC system ties together type III secretion and effector expression with effector translocase

Fig. 3. (a) Real-time RT-PCR showing the regulation of iron-utilization genes in the *qseE*, *qseG* and *qseF* mutant backgrounds. Significant downregulation of *fepE* and *entB* expression was seen. (b) Colony growth of mutant and complemented strains on media containing either 350 μ M DPD or 350 μ M DPD with 8 μ M haemin. Each strain was equally affected by DPD addition and able to recover equally to the WT when haemin was added. (c) Growth of mutant strains versus WT EHEC in DMEM. No growth defect was seen in any of the mutant strains. (d) Growth of mutant strains versus WT EHEC in DMEM containing 350 μ M DPD. The *qseE* and *qseG* mutants grew slightly more slowly than the WT, and this was complemented by the addition of the gene expressed on a plasmid. * $P \leq 0.05$ using Student's *t* test.

Fig. 4. QseEFG cross-talks with additional signalling systems. Real-time RT-PCR analysis was used to investigate the transcriptional regulation exerted by QseEFG on other two-component systems. Overall, expression of *phoP* and *phoQ* was downregulated in *qseE*, *qseF* and *qseG* mutants, while *rcsB* and *rcsC* were upregulated in the mutant strains. * $P \leq 0.05$; ** $P \leq 0.005$ using Student's *t* test.

tion. This suggests a very finely tuned regulation of AE lesion formation by EHEC.

The *qseEFG* genes are present in both K-12 and EHEC, and regulate pathogenic phenotypes, such as AE lesion formation (Reading *et al.*, 2007), as well as metabolic genes shared between pathogenic and non-pathogenic strains of *E. coli*. This study expands on our knowledge of two-component system regulation, and provides yet another example of how pathogens exploit two-component systems to regulate virulence.

ACKNOWLEDGEMENTS

We thank Lary Reitzer for suggestions on the nitrogen-utilization studies. The project described was supported by grant number AI053067 from the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases (NIAID). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIAID.

REFERENCES

- Anonymous (1997).** *Applied Biosystems Prism 7700 Sequence Detection System: User Bulletin #2*. Norwalk, CT: Perkin-Elmer Corp.
- Bader, M. W., Sanowar, S., Daley, M. E., Schneider, A. R., Cho, U., Xu, W., Klevit, R. E., Le Moual, H. & Miller, S. I. (2005).** Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* **122**, 461–472.
- Bolstad, B. M., Irizarry, R. A., Astrand, M. & Speed, T. P. (2003).** A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**, 185–193.
- Campellone, K. G., Robbins, D. & Leong, J. M. (2004).** EspFU is a translocated EHEC effector that interacts with Tir and N-WASP and promotes Nck-independent actin assembly. *Dev Cell* **7**, 217–228.
- Cheng, H. C., Skehan, B. M., Campellone, K. G., Leong, J. M. & Rosen, M. K. (2008).** Structural mechanism of WASP activation by the enterohaemorrhagic *E. coli* effector EspFU. *Nature* **454**, 1009–1013.
- Clemmer, K. M. & Rather, P. N. (2007).** Regulation of *flhDC* expression in *Proteus mirabilis*. *Res Microbiol* **158**, 295–302.
- Deng, W., Puente, J. L., Gruenheid, S., Li, Y., Vallance, B. A., Vázquez, A., Barba, J., Ibarra, J. A., O'Donnell, P. & other authors (2004).** Dissecting virulence: systematic and functional analyses of a pathogenicity island. *Proc Natl Acad Sci U S A* **101**, 3597–3602.
- Elliott, S. J., Wainwright, L. A., McDaniel, T. K., Jarvis, K. G., Deng, Y. K., Lai, L. C., McNamara, B. P., Donnenberg, M. S. & Kaper, J. B. (1998).** The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69. *Mol Microbiol* **28**, 1–4.
- Garmendia, J., Phillips, A. D., Carlier, M. F., Chong, Y., Schüller, S., Marches, O., Dahan, S., Oswald, E., Shaw, R. K. & other authors (2004).** TccP is an enterohaemorrhagic *Escherichia coli* O157:H7 type III effector protein that couples Tir to the actin-cytoskeleton. *Cell Microbiol* **6**, 1167–1183.
- Griffin, P. M., Ostroff, S. M., Tauxe, R. V., Greene, K. D., Wells, J. G., Lewis, J. H. & Blake, P. A. (1988).** Illnesses associated with *Escherichia coli* O157:H7 infections. A broad clinical spectrum. *Ann Intern Med* **109**, 705–712.
- Groisman, E. A. & Mouslim, C. (2006).** Sensing by bacterial regulatory systems in host and non-host environments. *Nat Rev Microbiol* **4**, 705–709.
- Hantash, F. M. & Earhart, C. F. (2000).** Membrane association of the *Escherichia coli* enterobactin synthase proteins EntB/G, EntE, and EntF. *J Bacteriol* **182**, 1768–1773.
- Hoch, J. A. (2000).** Two-component and phosphorelay signal transduction. *Curr Opin Microbiol* **3**, 165–170.
- Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U. & Speed, T. P. (2003).** Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264.
- Jarvis, K. G., Giron, J. A., Jerse, A. E., McDaniel, T. K., Donnenberg, M. S. & Kaper, J. B. (1995).** Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. *Proc Natl Acad Sci U S A* **92**, 7996–8000.
- Jerse, A. E., Yu, J., Tall, B. D. & Kaper, J. B. (1990).** A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci U S A* **87**, 7839–7843.
- Kaper, J. B., Nataro, J. P. & Mobley, H. L. (2004).** Pathogenic *Escherichia coli*. *Nat Rev Microbiol* **2**, 123–140.
- Kenny, B., DeVinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. & Finlay, B. B. (1997).** Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* **91**, 511–520.
- Laub, M. T. & Goulian, M. (2007).** Specificity in two-component signal transduction pathways. *Annu Rev Genet* **41**, 121–145.
- McDaniel, T. K., Jarvis, K. G., Donnenberg, M. S. & Kaper, J. B. (1995).** A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci U S A* **92**, 1664–1668.
- Mizuno, T. (1997).** Compilation of all genes encoding two-component phosphotransfer signal transducers in the genome of *Escherichia coli*. *DNA Res* **4**, 161–168.
- Murray, G. L., Attridge, S. R. & Morona, R. (2003).** Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz. *Mol Microbiol* **47**, 1395–1406.
- Ninfa, A. J. & Atkinson, M. R. (2000).** PII signal transduction proteins. *Trends Microbiol* **8**, 172–179.
- Ninfa, A. J. & Jiang, P. (2005).** PII signal transduction proteins: sensors of α -ketoglutarate that regulate nitrogen metabolism. *Curr Opin Microbiol* **8**, 168–173.
- Ninfa, A. J., Jiang, P., Atkinson, M. R. & Peliska, J. A. (2000).** Integration of antagonistic signals in the regulation of nitrogen assimilation in *Escherichia coli*. *Curr Top Cell Regul* **36**, 31–75.
- Otto, K. & Silhavy, T. J. (2002).** Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway. *Proc Natl Acad Sci U S A* **99**, 2287–2292.
- Ozenberger, B. A., Nahlik, M. S. & McIntosh, M. A. (1987).** Genetic organization of multiple *fep* genes encoding ferric enterobactin transport functions in *Escherichia coli*. *J Bacteriol* **169**, 3638–3646.
- Reading, N. C., Torres, A. G., Kendall, M. M., Hughes, D. T., Yamamoto, K. & Sperandio, V. (2007).** A novel two-component signaling system that activates transcription of an enterohemorrhagic *Escherichia coli* effector involved in remodeling of host actin. *J Bacteriol* **189**, 2468–2476.
- Reading, N. C., Rasko, D. A., Torres, A. G. & Sperandio, V. (2009).** The two-component system QseEF and the membrane protein QseG

link adrenergic and stress sensing to bacterial pathogenesis. *Proc Natl Acad Sci U S A* **106**, 5889–5894.

Reichenbach, B., Gopel, Y. & Gorke, B. (2009). Dual control by perfectly overlapping σ^{54} - and σ^{70} - promoters adjusts small RNA GlnY expression to different environmental signals. *Mol Microbiol* **74**, 1054–1070.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Smith, J. N., Dyszel, J. L., Soares, J. A., Ellermeyer, C. D., Altier, C., Lawhon, S. D., Adams, L. G., Konjufca, V., Curtiss, R., III & other authors (2008). SdiA, an *N*-acylhomoserine lactone receptor, becomes active during the transit of *Salmonella enterica* through the gastrointestinal tract of turtles. *PLoS One* **3**, e2826.

Throup, J. P., Koretke, K. K., Bryant, A. P., Ingraham, K. A., Chalker, A. F., Ge, Y., Marra, A., Wallis, N. G., Brown, J. R. & other authors (2000). A genomic analysis of two-component signal transduction in *Streptococcus pneumoniae*. *Mol Microbiol* **35**, 566–576.

Tobe, T., Ando, H., Ishikawa, H., Abe, H., Tashiro, K., Hayashi, T., Kuhara, S. & Sugimoto, N. (2005). Dual regulatory pathways integrating the RcsC–RcsD–RcsB signalling system control enterohaemorrhagic *Escherichia coli* pathogenicity. *Mol Microbiol* **58**, 320–333.

Tobe, T., Beatson, S. A., Taniguchi, H., Abe, H., Bailey, C. M., Fivian, A., Younis, R., Matthews, S., Marches, O. & other authors (2006). An extensive repertoire of type III secretion effectors in *Escherichia coli* O157 and the role of lambdoid phages in their dissemination. *Proc Natl Acad Sci U S A* **103**, 14941–14946.

Yamamoto, K., Hirao, K., Oshima, T., Aiba, H., Utsumi, R. & Ishihama, A. (2005). Functional characterization *in vitro* of all two-component signal transduction systems from *Escherichia coli*. *J Biol Chem* **280**, 1448–1456.

Zahrt, T. C. & Deretic, V. (2001). *Mycobacterium tuberculosis* signal transduction system required for persistent infections. *Proc Natl Acad Sci U S A* **98**, 12706–12711.

Edited by: R. J. Lamont