ChIP-seq and transcriptome analysis of the OmpR regulon of *Salmonella enterica* serovars Typhi and Typhimurium reveals accessory genes implicated in host colonization

Timothy T. Perkins,1 Mark R. Davies,1 Elizabeth J. Klemm,1 Gary Rowley,2 Thomas Wileman,1 Keith James,1 Thomas Keane,1 Duncan Maskell,3 Jay C. D. Hinton,4 Gordon Dougan1 and Robert A. Kingsley1*

1The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK.
2School of Biological Sciences, University of East Anglia, Norwich NR4 7TT, UK.
3Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK.
4Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, UK.

Summary

OmpR is a multifunctional DNA binding regulator with orthologues in many enteric bacteria that exhibits classical regulator activity as well as nucleoid-associated protein-like characteristics. In the enteric pathogen *Salmonella enterica*, using chromatin immunoprecipitation of OmpR:FLAG and nucleotide sequencing, 43 putative OmpR binding sites were identified in *S. enterica* serovar Typhi, 22 of which were associated with OmpR-regulated genes. Mutation of a sequence motif (TGTWACAW) that was associated with the putative OmpR binding sites abrogated binding of OmpR:6×His to the *tviA* upstream region. A core set of 31 orthologous genes were found to exhibit OmpR-dependent expression in both *S. Typhi* and *S. Typhimurium*. *S. Typhimurium*-encoded orthologues of two divergently transcribed OmpR-regulated operons (SL1068–71 and SL1066–67) had a putative OmpR binding site in the inter-operon region in *S. Typhi*, and were characterized using in vitro and in vivo assays. These operons are widely distributed within *S. enterica* but absent from the closely related *Escherichia coli*. SL1066 and SL1067 were required for growth on N-acetylmuramic acid as a sole carbon source. SL1068–71 exhibited sequence similarity to sialic acid uptake systems and contributed to colonization of the ileum and caecum in the streptomycin-pretreated mouse model of colitis.

Introduction

OmpR is a DNA binding protein that, with the cognate sensor EnvZ, co-ordinates transcriptional response to environmental factors including osmotic stress in many enteric bacteria (Forst and Roberts, 1994). OmpR/EnvZ are central to the adaptive response to the intestinal environment (Giraud et al., 2008), in part because of the distinct osmolyte composition of the lumen. As many as 125 genes in *Escherichia coli* (Oshima et al., 2002) and 305 genes in *Salmonella Typhi* (Perkins et al., 2009) have been implicated in OmpR/EnvZ-dependent expression. The OmpR regulon includes genes from the ‘ancestral’ core genome shared with many enteric bacteria as well as genes of the accessory genome. The latter include virulence-associated loci such as the *viaB* locus that encodes Vi polysaccharide biosynthesis genes, and genes encoded on *Salmonella* pathogenicity island 2 (SPI-2) via its regulation of *ssrAB* (Pickard et al., 1994; Feng et al., 2003; Perkins et al., 2009). OmpR-regulated orthologues in diverse enteric bacteria define the ancestral regulon and include porin genes such as *ompF* and *ompC*. However, the OmpR regulon exhibits considerable plasticity and can include genes of the ancillary genome acquired by horizontal gene transfer, many of which are involved in host–pathogen interactions. The acquisition of such genes and the ability to express them appropriately on moving from the intestinal lumen to the intracellular compartment were likely key features in the evolution of *Salmonella* (Bäumler, 1997; Groisman and Ochman, 1997).

The genus *Salmonella* consists of more than 2500 serotypes that exhibit diverse host range and pathogenicity (Bäumler et al., 1997; Popoff et al., 2004). Most of the >2500 serovars of *Salmonella enterica* have a relatively broad host range and are typically associated with gastroenteritis in human (Santos et al., 2001). In contrast, *S. enterica* serovar Typhi (*S. Typhi*) is highly host-adapted to cause the systemic disease typhoid specifically in...
human. S. Typhi can invade the intestinal mucosa but colonization of the intestine is relatively transient and rapid systemic dissemination can follow leading to typhoid. This distinct pathogenesis is driven at least in part by horizontally acquired genes that are required for virulence, including the OmpR-regulated viaB, encoding the Vi polysaccharide antigen (Pickard et al., 1994). The OmpR regulon includes both Salmonella pathogenicity island 1 (SPI-1) and SPI-2, mediated through ssrAB expression. The integration of such horizontally acquired genes into existing regulons is a recurring theme in the evolution of pathogenesis. The mechanism by which OmpR regulates gene expression is not fully understood. It has been proposed that OmpR has only weak specificity for DNA binding (Head et al., 1998; Rhee et al., 2008) and that it may have both a classical site-specific impact on gene expression through recruitment of RNA polymerase and additional nucleoid-associated protein (NAP)-like properties that may also impact global gene expression (Cameron and Dorman, 2012).

In this study we combine RNA-seq and ChIP-seq together with in vitro and in vivo phenotyping to define the interaction of OmpR with the chromosome and characterize two novel OmpR-regulated operons that are part of the S. enterica ancillary genome.

## Results

### Identification of candidate S. Typhi genes regulated by OmpR using ChIP-seq

We recently characterized the OmpR regulon of S. Typhi BRD948 using DNA microarray and RNA-seq (Perkins et al., 2009) (Table S1) identifying 208 genes by microarray and 305 genes by RNA-seq, that exhibited OmpR-dependent transcription during mid-log phase culture in rich media. In order to further characterize the OmpR regulon we used ChIP-seq to identify candidate genome regions that are preferentially associated with the OmpR protein in the S. Typhi genome. To this end a S. Typhi BRD948 derivative TT53.8 was constructed in which the 3′ end of ompR harboured an in-frame fusion with sequence encoding three repeats of the FLAG epitope (3×FLAG tag). TT53.8 expressed the fusion protein (OmpR:3×FLAG) in place of the wild-type OmpR protein from the native chromosomal location at single copy. To assess if this fusion protein had comparable function to wild-type OmpR, we indirectly monitored the expression of the OmpR-dependent viaB locus in TT53 (Pickard et al., 1994). Agglutination of S. Typhi TT53 with anti-Vi antiserum was indistinguishable to that of S. Typhi BRD948 in low-salt and high-salt culture media (data not shown).

Salmonella Typhi TT53.8 or BRD948 were grown to mid-log phase (OD600 = 0.6) and ChIP-seq was performed on DNA precipitated by anti-FLAG antibody. The normalized sequence depth at each base of the reference genome sequence was plotted as the number of standard deviations from the mean (z-score) to identify regions of significantly enriched sequence coverage (z-score > 3) and 43 ChIP-enriched peaks that were within intergenic regions were studied further (15 lay within annotated coding sequence and were excluded from further analysis) (Fig. 1, Table S2).

Twenty-two of the genes with a sequence enrichment peak in their upstream region also exhibited OmpR-dependent expression as determined by RNA-seq or microarray analysis (Perkins et al., 2009) (Fig. 1, Table 1). These included many previously identified OmpR-regulated genes such as tviA and ompS1 (Fig. 2). Some genes associated with aerobic lifestyle such as citrate synthase (gltA) and succinate dehydrogenase C (sdhC) also contained enrichment peaks. A ChIP peak was identified in the intergenic region of the divergently transcribed operons encoding stdA and dppA. Another within the intragenic region of two divergently transcribed putative operons encoding genes t1787–1790 and t1791–93 (Table 2). Surprisingly, statistically significant peaks with a z-score > 3 were not identified in the well-characterized OmpR-regulated genes ompF and ompC, although a minor peak that fell just short of the statistical cut-off, mapped extensively to motifs (C1–3) implicated in OmpR binding (Fig. 2C). To determine if the C-terminal FLAG tag of OmpR impacted binding to the ompC or ompF promoter region we compared expression of these genes in the wild-type (BRD948) and ompR:FLAG strains (TT53.8) (Fig. S1). Expression of tviA and ompF was similar in these two strains but ompC was expressed at a significantly lower level in TT53.8. The degree to which ompC expression was decreased in TT53.8 compared to wild-type BRD948 was not as pronounced as that in a strain in which ompR was deleted (TT10) suggesting that some OmpR activity for the ompC promoter was retained in the epitope-tagged protein.

### Identification of nucleotide sequence motifs associated with OmpR binding

To identify sequence motifs within the 43 intergenic ChIP-enriched sequence coverage peaks that may be involved in OmpR binding, nucleotide sequences were compared using the YMF algorithm (Sinha and Tompa, 2003), which identifies candidate binding sites by searching for statistically overrepresented motifs. Five eight-nucleotide motifs (z-score > 9.8, Table S2) were identified in 14 separate loci, with some loci containing multiple motifs. The motif TGTWACAW occurred 21 times, in 12 ChIP-enriched sequences including the viaB locus (S′ tviA) where it precisely coincided with the peak of sequence enrichment.
Fig. 1. Circular plot of the S. Typhi Ty2 genome indicating ChIP-seq coverage and the position of OmpR-regulated genes. Concentric circular tracks indicate: (a) plot of the sequence coverage expressed as the number of standard deviations from the mean (z-score) with values > 0 (red) or < 0 (blue); (b) differentially regulated genes identified by microarray (Perkins et al., 2009); (c) significant ChIP-seq peaks defined as spanning ≥ 36 bp and with sequence read depth ≥ 3 standard deviations (z-score) above the mean; (d) S. Typhi genes with colour coding indicating function: dark blue, pathogenicity/adaptation; black, energy metabolism; red, information transfer; dark green, membranes/surface structures; purple, degradation of small molecules; yellow, central/intermediary metabolism; light blue, regulators; pink, phage/IS elements; orange, conserved hypothetical; pale green, unknown function; brown, pseudogenes; (e) genes exhibiting OmpR-dependent expression that have a ChIP-seq peak in 5′ UTR region; (f) ChIP-seq peaks associated with differentially regulated genes; and (g) S. Typhi genes that exhibited OmpR-dependent expression in both microarray and RNA-seq experiments (Perkins et al., 2009).

Table 1. S. Typhi genes that exhibit OmpR-dependent expression and have candidate OmpR binding sites in their 5′ UTR determined by ChIP-seq.

| Gene    | Ty2 ID | CT18 ID | RNA-seq*   | P-value | Microarray* | P-value | Ty2 annotation                      |
|---------|--------|---------|------------|---------|-------------|---------|-------------------------------------|
| ackA    | 10527  | STY2567 | 0.56       | 2.6 × 10⁻² | NS          | NS      | Acetate kinase                      |
| mglB    | 10665  | STY2424 | 2.0        | 9.4 × 10⁻³ | 2.17        | 3.3 × 10⁻⁴ | D-galactose binding periplasmic protein |
| ompS1   | 10883  | STY2203 | 3.0 × 10⁻¹ | 7.9 × 10⁻¹ | 4.2 × 10⁻¹ | 3.7 × 10⁻⁴ | Outer membrane protein S1         |
| stdA    | 10918  | STY2167 | NS         | NS      | 1.9         | 1.6 × 10⁻³ | Flagellin                          |
| ssrA    | 11260  | STY1728 | 9.9 × 10⁻² | 1.6 × 10⁻² | NS          | NS      | Putative two-component sensor kinase |
| −       | 11790  | STY1167 | 1.0 × 10⁻¹ | 3.1 × 10⁻² | 8.3         | 7.1 × 10⁻⁴ | Hypothetical protein               |
| −       | 11791  | STY1166 | 8.18       | 9.1 × 10⁻² | 1.8         | 3.3 × 10⁻⁵ | N-acetylmannosamine-6-phosphate 2-epimerase |
| ompX    | 12055  | STY0872 | 4.4 × 10⁻¹ | 3.6 × 10⁻² | NS          | NS      | Outer membrane protein X           |
| sdhC    | 12144  | STY0775 | NS         | NS      | 4.9         | 9.3 × 10⁻¹⁰ | Succinate dehydrogenase            |
| gtaA    | 12146  | STY0773 | NS         | NS      | 6.8         | 6.4 × 10⁻¹⁰ | Citrate synthase                   |
| stdA    | 12940  | STY3177 | 2.20 × 10⁻¹ | 3.4 × 10⁻² | NS          | NS      | Probable fimbrial protein          |
| −       | 13197  | STY3460 | 5.21 × 10⁻¹ | 9.3 × 10⁻² | NS          | NS      | Tryptophan permease                |
| udp     | 13329  | STY3591 | 3.8        | 7.5 × 10⁻² | NS          | NS      | Uridine phosphorylase              |
| purH    | 13455  | STY3709 | 3.9 × 10⁻¹ | 2 × 10⁻²  | NS          | NS      | Bifunctional phosphoribosylaminomimidazolylcarboxamide formyltransferase |
| rplK    | 13478  | STY3736 | 4.3 × 10⁻¹ | 6.5 × 10⁻³ | NS          | NS      | 50S ribosomal protein L11          |
| typA    | 13613  | STY3871 | 3.8 × 10⁻¹ | 8.2 × 10⁻² | NS          | NS      | GTP binding protein                |
| −       | 13871  | STY4154 | 2.2        | 9.4 × 10⁻² | NS          | NS      | Putative transcriptional regulator |
| dppA    | 13885  | STY4168 | 2.2        | 7.7 × 10⁻² | 2.1         | 3.9 × 10⁻⁷ | Periplasmic dipeptide transport protein |
| ompr    | 14004  | STY4294 | 1.1 × 10⁻¹ | 6.3 × 10⁻³ | 3.6 × 10⁻¹ | 1.9 × 10⁵ | Osmolarity response regulator      |
| −       | 14353  | STY4662 | 3.8 × 10⁻³ | 1 × 10⁻⁴  | 3.8 × 10⁻² | 4.7 × 10⁻¹⁰ | Vi polysaccharide biosynthesis protein |
| −       | 14354  | STY4663 | 2.0 × 10⁻¹ | 2.3 × 10⁻² | NS          | NS      | Hypothetical protein               |
| −       | 14357  | STY4666 | 4.4 × 10⁻¹ | 8.4 × 10⁻³ | NS          | NS      | Probable phage integrase           |

a. RNA-seq data, normalized ratio (WT : ΔompR) of sequence coverage determined by Illumina GA11 sequencing (Perkins et al., 2009).

b. Normalized ratio (WT : ΔompR) of microarray (n = 3) fluorescent intensities from three biological replicates hybridized on four microarrays (Perkins et al., 2009).

NS, not significantly different.
motif was present in two copies in the upstream region of seven genes: *ompS1*, *csgD*, *sdhC*, *gaiP*, *dppA*, *pckA* and *t4357*. t4357 encodes a putative integrase encoded on a prophage – 3.6 kbp upstream of *tviA*. A second motif AYGGCCCTA was present in single copy in the upstream region of four loci: *t0528*, *t1320*, *dppA* and *tviA*. There was also a significant difference in the magnitude of ChIP sequence peaks containing motif AYGGCCCTA compared with those with no identifiable motif (Student’s *t*-test, *P* < 0.0001) (Fig. S2), suggesting a link between this sequence and the avidity of OmpR binding. None of the sequence motifs are present in the previously described C1–3 or F1–4 OmpR binding sites in *ompC* and *ompF* promoter regions respectively.

The largest enrichment peak (*z*-score = 16.23) was found upstream of *tviA* of the *viaB* locus, encoding four different candidate motifs: TGTWACAW, CTAGACTA, AYGGCCCTA and AACTAACW (Table S2a). To find if the TGTWACAW motif was involved in binding of OmpR to the *tviA* upstream region, we used an electrophoresis mobility shift assay (EMSA) with phosphorylated recombinant OmpR::6×His protein and oligonucleotide probes. The probes comprised either *tviA* –133 to –460 or *tviA* –303 to –377 of the *tviA* upstream region. Arbitrary mutation of the TGTTACAA motif in the upstream region also coincides with the genome sequence most highly overrepresented following ChIP enrichment (*z*-score = 30, Fig. 2).

**The OmpR regulons of *S. Typhi* and *S. Typhimurium* contain a core set of shared orthologous genes**

We next compared the previously unreported OmpR regulon of the broad host range *S. Typhimurium* strain SL1344 with that of the human restricted *S. Typhi* Ty2 (Perkins *et al.*, 2009) to identify previously uncharacterized genes, controlled by OmpR in both pathogens. A total of 208 genes and 329 genes were expressed in an OmpR-dependent manner in *S. Typhi* and *S. Typhimurium* respectively. Of these, 31 orthologous genes were expressed in an OmpR-dependent manner in both serotypes (Table S3). OmpR-dependent expression levels of genes that were OmpR-regulated in both *S. Typhi* and *S. Typhimurium* showed a high degree of correlation (Fig. 4; *R*² = 0.73) indicating strongly conserved regulation between the two serovars in this cohort of genes. OmpR-dependent genes found in both serotypes included *ompS1*, *ompC*, *sprB* and *ompR*, all of which showed decreased expression in the absence of OmpR. A number

---

**Fig. 2.** ChIP-seq coverage mapped to the *S. Typhi* Ty2 genome sequence in OmpR ChIP-seq. The *z*-score (number of standard deviations from the mean) is plotted at each base of the genome sequence in the 5′ UTR of (A) *tviA*, (B) *ompS1*, (C) between *csgB* and *csgD* and (D) t1787–1793. Motifs identified in this manuscript are indicated (grey boxes) labelled ‘A’ (TGTWACAW), ‘B’ (CTAGACTA), ‘C’ (AYGGCCCTA), ‘D’ (AACTAACW) and ‘E’ (ACTTAGCS). OmpR binding sites in the *ompC* upstream region are indicated (open boxes) and labelled C1–3.
of genes were upregulated in the absence of OmpR, including the succinate dehydrogenase genes sdhCDA (Cunningham and Guest, 1998), fatty acid dehydrogenase genes fadABI (Campbell et al., 2003), narK (Rowe et al., 1994), required for nitrite extrusion, and the nitrite reductase gene nrfA (Clarke et al., 2008).

Potentially important differences in the expression of SPI-1, SPI-2 and flagellin secretion apparatus in S. Typhi and S. Typhimurium were also revealed by the transcriptomic data in S. Typhimurium; 28 SPI-1-associated genes exhibited decreased expression in the absence of OmpR, including the sprB gene. Furthermore, several genes associated with the flagella type III secretion system (flGHJLMO) and 10 apparatus genes encoded on SPI-2 (ssaA, ssaB, ssaGHIJKLT and STM1410) also exhibited up to fivefold greater expression in the absence of a functional OmpR (Table S4). In contrast, the only known SPI-1 gene that was OmpR-dependent in S. Typhi was sprB, and this encodes a transcriptional regulator (Golubeva et al., 2012). However, it is important to note that the culture conditions employed in our studies are known to result in low expression of SPI-1 and SPI-2 genes and therefore the biological impact of the observed differences in OmpR regulation in these conditions is not clear.

Table 2. Novel OmpR-regulated genes in S. Typhi operons t1787–90 and t1791–93 and S. Typhimurium orthologues.

| S. Typhi Ty2 ID | S. Typhimurium orthologue (SL1344) | Log2 fold change ompR/+ompR | S. Typhi* | GC content (%) | Putative function |
|----------------|-----------------------------------|-----------------------------|-----------|---------------|-----------------|
| t1787          | SL1071                            | 4.03                        |           | 46.8          | Oxidoreductase   |
| t1788          | SL1070                            | 2.86                        |           | 45.1          | Sialic acid transporter |
| t1789          | SL1069                            | 4.44                        |           | 36.3          | Secreted protein |
| t1790          | SL1068                            | 8.33                        |           | 40.2          | Sialic acid lyase |
| t1791          | SL1067                            | 1.78                        |           | 51.4          | N-acetylmannosamine-6-P epimerase |
| t1792          | SL1066                            | 1.33                        |           | 43.9          |                 |
| t1793          |                                   | 1.21                        |           | 47.0          | SSS sialic acid transporter |

a. Log2 fold change in transcript abundance determined by RNA-seq (Perkins et al., 2009).

Fig. 3. Electrophoretic mobility shift assay to determine binding of recombinant OmpR::6x:His to the tvIA upstream region. FAM-labelled double-stranded DNA fragments corresponding to the −133 to −459 (A) and −303 to −377 (B) upstream region of tvIA were mixed with increasing concentrations of phosphorylated recombinant OmpR::6x:His protein and mobility monitored by electrophoresis on a 15% native polyacrylamide gel.

Fig. 4. Comparison of OmpR-dependent expression of orthologous genes in S. Typhi and S. Typhimurium in microarray experiments. Fold change in expression of orthologous genes in wild-type (WT) compared to ΔompR strains of S. Typhi Ty2 and S. Typhimurium strain SL1344 are indicated (black circles). Orthologous genes t1789/SL1069 highlighted (yellow circle). The linear regression of the data points (solid black line, R² = 0.73) and the line of equivalence (broken grey line) are indicated.

© 2012 Blackwell Publishing Ltd, Molecular Microbiology, 87, 526–538
t1787–t1790 and t1791–1793 encode putative nutrient-scavenging systems

We next characterized the function of t1787–t1790 and t1791–1793 using a number of in vitro and in vivo assays. In S. Typhimurium SL1344, genes SL1071–SL1068 (STM1133–STM1130) and SL1067–SL1066 (STM1129–STM1128) are orthologues of the S. Typhi t1787–t1790 and t1791–1793 genes respectively (Fig. 5). However, t1792 and t1793 of S. Typhi are present as a single open reading frame (SL1066) in S. Typhimurium, suggesting that these genes may represent fragments of a pseudo-gene in S. Typhi.

We considered that these operons may be involved in host–pathogen interactions since they were absent from the closely related species E. coli (strain K12) (Blattner et al., 1997) but were highly conserved within S. enterica serotypes exhibiting > 99% identity at the amino acid level in these pathogens (Fig. 5). The transcriptomic data showed that expression of at least two of the S. Typhimurium orthologues was OmpR-dependent, namely SL1068 (4.75-fold increase, \( P < 0.05 \), orthologue of t1791) and SL1069 (9.59-fold increase, \( P < 0.05 \), orthologue of t1789, Fig. 4). Proteins encoded by several of the genes in these operons exhibit similarity to proteins that have previously been implicated in sialic acid uptake and metabolism. An orthologue of SL1066 encoded by S. Typhimurium LT2 (STM1128) is a sodium solute symporter (SSS) family transporter of sialic acid and shares 44–48% identity at the amino acid level with similar transport systems in Lactobacillus spp. and Staphylococcus spp. (Severi et al., 2010). SL1067 is a homologue of nanE (SL3309) that is encoded elsewhere on the SL1344 chromosome, sharing 69% identity at the amino acid level. SL1068 to SL1071 have previously been proposed to be orthologues of genes encoded by E. coli K12 (nanM, nanC, yjH and yhC respectively), some of which have been implicated in sialic acid metabolism (Severi et al., 2008). However, the genomic context for these genes is quite different in E. coli compared to S. Typhimurium and amino acid sequence identity ranges from just 22% for NanC to 62% for YhjC, considerably less than observed for orthologous proteins of E. coli and S. Typhimurium of approximately 90% (Parkhill et al., 2001). We therefore tested the hypothesis that genes within these operons were involved in utilization of acetylated amino sugars including sialic acid as a sole carbon source, and colonization of the murine host.
To this end, we determined the ability of *S. Typhimurium* SL1344 and the isogenic mutant derivatives RAK103 (ΔSL1067/SL1066) and RAK105 (ΔSL1068–SL1071) to grow on M9 minimal media supplemented with one of several alternative acetylated amino sugars as the sole source of carbon. RAK103 (ΔSL1067/SL1066) was deficient in growth on N-acetylmuramic acid, a component of peptidoglycan, but grew normally on all other sole carbon sources tested (Table 3). A strain (SW738) in which SL1067/SL1066 were reintroduced onto the chromosome of strain RAK103 (ΔSL1067/SL1066) by phage-mediated transduction grew normally on N-acetylmuramic acid as a sole carbon source. No defect in growth on any of the carbon sources tested was observed for RAK105 including N-acetylneuraminic acid, a common sialic acid, possibly because this strain encodes a second sialic transport protein, NanT.

Experimental infections of mice with *S. Typhimurium* are a common surrogate model for typhoid fever (Tsolis *et al.*, 1999). To determine if these OmpR-regulated genes contribute to the pathogenesis of *S. Typhimurium* in mice we carried out competitive infection experiments between the fully virulent *S. Typhimurium* RAK113 and mutant derivatives harbouring deletions within each operon (RAK105 ΔSL1068–SL1071, orthologues of t1787–t1790) and RAK103 (ΔSL1067/SL1066, orthologues of t1791–3) (Table 4). Initially, groups of C57BL/6 mice were inoculated orally with $1 \times 10^8$ cfu containing an equal mixture of either RAK103 (ΔSL1067/SL1066, orthologues of t1791–3) or RAK105 (ΔSL1068–SL1071, orthologues of t1787–1790) and RAK113. No significant difference in the ratio of each derivative was observed in the caecum, ileum, Peyers patch, spleen or liver (Table 4).

Since *S. Typhimurium* normally causes inflammatory diarrhoea manifesting as gastroenteritis we determined if these genes are required for colonization and growth during a robust inflammatory response in the intestinal tract. To this end we performed mixed inoculum experi-

---

### Table 3. Growth of *S. Typhimurium* RAK113 (wild-type) or strains RAK103 and RAK105 on M9 minimal media agar supplemented with alternative sole carbon sources.

| Carbon source             | Wild type | ΔSL1067–SL1066 | ΔSL1068–SL1071 |
|---------------------------|-----------|----------------|----------------|
| No carbon source          | --        | --             | --             |
| Glucose                   | ++        | ++             | ++             |
| Pectin                    | --        | --             | --             |
| Galacturonic acid         | --        | --             | --             |
| Muramic acid              | --        | --             | --             |
| Cytidine sialic acid      | +         | +              | +              |
| N-acetylmuramic acid      | ++        | --             | ++             |
| N-acetylneuraminic acid   | ++        | ++             | ++             |

The surface of the agar was inoculated with each strain cultured on LB agar and incubated for 48 h at 37°C. Growth was assessed and recorded as no growth (–), growth retarded relative to that with glucose as a sole carbon source (+) and comparable growth to that with glucose as a sole carbon source (++)..

---

### Table 4. Competitive infection of C57BL/6 mice.

|                  | Caecum | Ileum | Mesenteric lymph nodes | Liver | Spleen | Mesenteric lymph nodes | Liver | Spleen |
|------------------|--------|-------|------------------------|-------|--------|------------------------|-------|--------|
|                  | Log10 ratio | SE | Log10 ratio | SE | Log10 ratio | SE | Log10 ratio | SE | Log10 ratio | SE |
| Typhoid infection model | WT : SL1068–71 | 0.26 | 1.40 | 0.55 | 1.25 | 0.65 | 0.80 | 0.02 | 0.33 | 0.33 | 0.15 |
|                  | RAK113 | 0.39 | 0.12 | 0.14 | 0.16 | 0.36 | 0.26 |
| RAK105 complemented | WT : SL1068–71 | 0.39 | 0.12 | 0.14 | 0.16 | 0.36 | 0.26 | 0.08 | 0.20 | 0.23 | 0.18 |
|                  | RAK103 | 0.08 | 0.20 | 0.23 | 0.18 | 0.23 | 0.18 | 0.23 | 0.18 | 0.23 | 0.18 |

For the typhoid infection model, groups of five C57BL/6 mice were inoculated with $1 \times 10^8$ cfu containing an equal mixture of either RAK103 (ΔSL1067/SL1066, orthologues of t1791–3) or RAK105 (ΔSL1068–SL1071, orthologues of t1787–1790) and RAK113.

* P < 0.05, ** P < 0.005.

The data were calculated from the combined data from two independent experiments each of which individually had comparable outcomes.
ments in the streptomycin-pretreated mouse model of colitis (Hapfelmeier et al., 2004; Hapfelmeier and Hardt, 2005). Groups of streptomycin-pretreated mice were inoculated with $1 \times 10^7$ cfu of an equal mixture of $S$. Typhimurium RAK103 or RAK105 and RAK113. Four days post inoculation RAK103 was present in similar proportion to RAK113 (Table 4). However, RAK113 was present in approximately threefold greater numbers in the caecum of mice compared with RAK105 (Table 4). This decrease in fitness specifically in the inflamed gut was statistically significant, and reproducible. Furthermore, when the SL1067/SL1066 genes were reintroduced into RAK105 by phage-mediated transduction giving rise to strain SW771, the ability to compete successfully with the wild-type RAK113 in colonization of the caecum in streptomycin-pretreated mice was restored (Table 4).

Discussion

Transcriptional regulons have been defined using DNA microarrays and more recently by RNA-seq approaches. Observed changes in transcript abundance can be directly or indirectly related to a regulator protein binding either within an operator or at a secondary regulatory site. We have combined measurement of transcript abundance with a direct assay of OmpR binding using ChIP-seq to gain a more complete understanding of the regulon and identify novel genes within this network. Using this approach genome sequences that were enriched included many previously described OmpR-regulated genes (Fig. 1). The most highly enriched sequences were upstream of the $viaB$ locus ($tviA$) (Fig. 2A). Furthermore, there was considerable enrichment in the 5′ UTR of ompS1 (Fig. 2B), ompR and between the divergently transcribed csg operons. These observations provided proof of principle that the ChIP-seq approach identified well-known OmpR-regulated genes. Perhaps surprisingly, substantial enrichment peaks were not observed in the 5′ UTR of the ompC and ompF genes, even though these are known to be regulated by OmpR (Rhee et al., 2008). A minor peak that did map to previously identified OmpR binding sites (C1–3) was present, but fell below the criteria used for peak identification. The reason for the lack of enrichment peaks associated with the ompC and ompF genes is not known, but may be related to the specific culture conditions used in this study resulting in incomplete phosphorylation of OmpR or due to interference from the C-terminal FLAG epitope tag. The presence of a C-terminal FLAG epitope had little impact on expression of the $tviB$ and $ompF$ genes but the ompC gene exhibited decreased expression, suggesting that the epitope may impact binding sites differently. Therefore, it is possible that all OmpR binding sites were not identified in this study.

Specific binding of OmpR is thought to depend at least in part on short nucleotide sequence motifs in the 5′ UTR region of genes within the regulon (Huang et al., 1994; Harlocker et al., 1995; Rhee et al., 2008), although the dependence on specific sequence is markedly less pronounced than for another two-component regulator of $Salmonella$, PhoP (Harari et al., 2010). Specific recognition of these motifs by OmpR is dependent on the phosphorylation state of the regulator and subsequent positive regulation of transcription results from direct interaction with RNA polymerase. A number of motifs have been proposed based on sequence similarity in the 5′ UTR of the ompC and ompF genes of E. coli, and DNase footprint analysis. However, the lack of specificity for binding to these motifs is shown by the absence from the upstream sequence of other OmpR-regulated genes. We used the YMF algorithm to identify sequences that were statistically overrepresented within enriched sequences following immunoprecipitation. While no such motifs were identified in 28 of 43 enrichment peaks using this approach, the motif (TGTTWACAW) was present in 12 enrichment peaks and appeared in multiple copies (two or three copies) in seven of these regions. The motif TGTTTACAA was present precisely at the point of greatest ChIP enrichment in the $tviA$ upstream region determined by sequencing. Furthermore, this sequence was critical for binding of recombinant OmpR–6×His in vitro using an EMSA approach. A total of four additional motifs were also identified and generally where these were present they were in the 5′ UTR of genes that also contained the common motif TGTTWACAW. This suggested there may be a functional link between these sequences.

A total of 31 orthologous pairs of genes showed OmpR-dependent expression in both S. Typhi and S. Typhimurium. Many more genes were OmpR-dependent in either S. Typhi or S. Typhimurium. The reason for this distinction is not clear but may be related to differences in the phosphorylation state of EnvZ, the OmpR cognate sensor kinase, that has been reported between these two serotypes (Oropeza and Calva, 2009). OmpR has pleiotropic effects on the homeostasis of the bacterial cell and these may manifest differently in Typhi and Typhimurium due to the overall differences in genotype.

Facultative anaerobic bacteria such as E. coli and Salmonella are thought to occupy a niche in the mucus layer close to the intestinal epithelium. Here they scavenge monosaccharides produced from the hydrolysis of complex polysaccharides and dietary fibre by anaerobic bacterial members of the microbiota (Chang et al., 2004). However, pathogenic bacteria such as Salmonella (Stecher et al., 2007) can induce a strong inflammatory response that results in a decrease in the population of many components of the microbiota that not only alters
the available nutrients (Stecher et al., 2008) but also available respiratory electron acceptors (Winter et al., 2010). Two divergently transcribed operons that were differentially expressed on inactivation of the ompR gene and contained a candidate OmpR binding site were predicted to be involved in scavenging and transport of alternative carbon sources. The predicted product of t1787–t1790 (SL1071–SL1068) had sequence similarity to sialic acid transport system, such as NanA/NanT in S. Typhimurium (Plumbbridge and Virm, 1999). The proteins encoded by t1791–3 (SL1067–SL1066) are also predicted to be involved in sialic acid metabolism. SL1067 exhibited homology with NanE, an N-acetylmannosamine-6-phosphate epimerase, and SL1066 orthologue has been reported to complement a nanT mutant of E. coli for growth in sialic acid as a sole source of carbon (Severi et al., 2010). However, deletion of these genes did not detectably impact utilization of sialic acid in vitro, presumably because of the presence of nanT and nanE. However, deletion of these genes resulted in the inability to use a related acetylated carbon compound, N-acetylmuramic acid, as a sole source of carbon during culture in vitro. Furthermore, although SL1068–SL1071 were not obviously required for colonization of the murine host in conventional mixed inoculum experiments, in the colitis model there was a reproducible diminution of the murine host in conventional mixed inoculum experiments, in the colitis model there was a reproducible diminution of the murine host in conventional mixed inoculum experiments.

Sialic acid has several potential impacts on host–pathogen interactions. It can be utilized as a carbon or nitrogen source, and is used by Haemophilus influenzae to modify LPS in order to evade detection by the host immune system (Bertin et al., 2012). However, it is likely that nutrient availability is altered as a result of the inflammatory response induced by Salmonella during infection, concomitant with disturbance of the normal microbiota (Stecher et al., 2007). Indeed, it was recently reported that Salmonella can use host-derived ethanolamine as a carbon source and respiratory electron acceptor following the switch to anaerobic respiration in the inflamed intestine (Thiennimit et al., 2011). Our findings suggest that additional OmpR-regulated genes may contribute to nutrient scavenging in the inflamed intestine.

Experimental procedures

Bacterial culture and strains

Salmonella Typhi was cultured routinely in LB broth with aromatic amino acids and pABA supplements as described previously (Lowe et al., 1999). Growth media were supplemented with antibiotics as appropriate at final a concentration of 0.05 mg l⁻¹ kanamycin or 0.03 mg l⁻¹ chloramphenicol. A strain in which the ompR gene is replaced by the aph gene encoding kanamycin resistance has been described previously (Kingsley et al., 2003). To construct a chromosomally encoding ompR::3xFLAG, overlap extension PCR was employed to create a sequence encoding an in-frame 3xFLAG peptide at the C-terminus of the ompR gene. This was complicated by the overlapping start–stop codon of the ompR locus (Parkhill et al., 2001). The Shine–Dalgarno sequence of the envZ gene, predicted to be encoded in the ompR ORF, was encoded downstream of the stop codon after the 3xFLAG sequence. This sequence was cloned into the suicide vector pWT12 and the strain TTS3 made by allelic exchange (Turner et al., 2006). Primers used were as follows: CGTCAGGGAAAGGAGCTCC, 5’ to 3’ ompR bases (364.383), CCGTCATGTCCTTTGATAGCTGCTTTA GAAAGTCCAGGA (full reverse primer sequence 5’ to 3’), GACTCAGAAACATGCGTATGTTATATTAGATGGAAGCG GTTCTAAGGC [concatenated primers are 5’ to 3’ forward (1:69 FLAG + 1:20)], CGAAACGACGGGCGACG [reverse for envZ is 5’ to 3’ (213:230)]. A strain designated RAK105 in which the SL1068–SL1071 genes of SL1344 were replaced by the aph gene was constructed using oligonucleotides 5’ accataagctcaatagtgaagctggaa atacctgatcatactttaattatctgacg TGGTAGCTGAGCTTCTTAC 3’ and 5’ cataaggccagcggccacccg gccactaaaccttctacccg tggcgctgaaacttcagttactcata catcatatatatcattcatcactgacc 3’ to amplify with the pKD4 plasmid template. A strain designated RAK103 in which the SL1068–SL1071 genes of SL1344 were replaced by the aph gene was constructed using oligonucleotides 5’ cgcgtttggttcgcagctgtgctgtgtg tatactgctgtatgataa TGTTGAGCTGAGCTTCGTACG 3’ and 5’ agacataacataaaccgaaaactaattac gactgcatatgatattcatctcattag 3’ to amplify with the pKD4 plasmid template. In all cases the mutation was retransduced into S. Typhimurium SL1344 using bacteriophage P22 in order to decrease the chances of the accumulation unlinked mutations during the passaging of bacteria during mutation construction. Strains (SW738 and SW771) in which the wild-type copy of genes SL1066 and SL1067 or SL1068–1071 was replaced in strains RAK103 (ΔSL1066–1067::aph) and RAK105 (ΔSL1068–1071::aph), respectively, were constructed using phage-mediated transduction. In order to select for transductants in this region a cat gene was introduced in the intergenic region of SL1071 and SL1072 using oligonucleotide primers 5’ ggcaaggataaacatatcagctcatgact gcggctaaaatattggaagg 3’ and 5’ ccgctgtatatcataagccacgcggccacccg gccactaaaccttctacccg tggcgctgaaacttcagttactcata catcatatatatcattcatcactgacc 3’. The cat gene was then introduced into S. Typhimurium strain RAK105 by P22 transduc-
tion and chloramphenicol-resistant transductants selected on LB + Cm culture medium. Transductants that were resistant to chloramphenicol but sensitive to kanamycin were identified by replica plating on culture media containing the appropriate antibiotics. One such transductant was designated SW771 and the replacement of the aph gene with the wild-type SL1068–1071 confirmed by PCR amplification.

Expression analyses using microarray data and RNA-seq

Bacterial strains were cultured to OD_{600} = 0.6 and immediately fixed with RNAProtec (Qiagen) and harvested. The pellet was dried and RNA isolated using SV RNA isolation kit (Promega) according to manufacturer’s instructions; however, elutions were performed using DEPC-treated water (Ambion). Dye incorporation, microarray design and analysis were performed as described previously (Kelly et al., 2004). RNA-seq data were described previously (Perkins et al., 2009). For S. Typhimurium microarrays, strain SL1344 and variants were cultured shaking at 250 r.p.m. in a New Brunswick Innova 3100 water bath at 37°C in 25 ml of fresh LB medium inoculated with a 1:100 dilution from an overnight bacterial culture. Three biological replicates were performed for each strain, and RNA was extracted at an optical density at 600 nm of 0.6 (mid-exponential phase). RNA was extracted using Promega’s SV 96 total RNA purification kit. RNA quality was assessed on an Agilent 2100 Bioanalyser. Transcriptomic analyses were performed on a SALSA microarray that contained the 5000 open reading frames (ORFs) identified from the sequence of S. enterica serovar Typhimurium SL1344, as described previously (Balbontin et al., 2006). Hybridization, microarray scanning and data analysis were all performed as described previously (Kelly et al., 2004), using a false-discovery rate of 0.05. The expression data have been deposited in the NCBI Gene Expression Omnibus http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=pbkdfwskomsowp&q=acc=GSE35938 and are accessible through GEO Series Accession Number GSE35938. All microarray data are MIAME-compliant.

ChIP-seq

Salmonella Typhi ompR::3×FLAG (strain TT53) and S. Typhi BRD948 were cultured in LB broth to OD_{600} = 0.6, lysed, incubated with 1% formaldehyde at 37°C for 20 min to cross-link DNA with protein then quenched with glycine (pH7) to a final concentration of 0.5 M. Cells were harvested and washed twice in TBS and lysed by osmotic shock. Genomic DNA was then treated with pronase (0.8 mg ml^{−1}, Sigma) at 65°C overnight. The nucleotide sequence of genomic DNA fragments was determined by Illumina GAII paired-end sequencing with read length 36 bp and mapped to the S. Typhi Ty2 whole genome sequence (AE014613). Sequence data were mapped to the S. Typhi Ty2 genome using the same parameters as previously described (Perkins et al., 2009), without assigning the sequence reads to each strand. Plots were z-score-normalized, in order to indicate the number of standard deviations above or below the mean for each datum point, and the differences between the untagged S. Typhi Ty2 and ompR::3×FLAG-tagged associated DNA sequences determined. Plots were then read into the genome browser tool Artemis (Rutherford et al., 2000). The Peakfinder function was used to determine enrichment for OmpR::3×FLAG bound DNA sequences. The Peakfinder function (36 bp window and z-score cut-off score set to 3) identified 58 peaks. Due to the background noise of the mapped sequence data plots and low stringency of the Peakfinder conditions, identified peaks were then filtered manually. Sites of DNA enrichment present within a predicted or known CDS were ignored unless there were multiple similar sites nearby, reducing the total number of analysed peaks to 43. Enriched sequences were then input to the motif finding algorithm YMF with the length of motif set to eight nucleotides and with a maximum of two redundant bases (Sinha and Tompa, 2003).

RNA extraction, reverse transcription-PCR (RT-PCR) and real-time PCR

RNA was extracted from S. Typhi using a Fast RNA Blue Kit (MP Biomedicals) according to the instructions of the manufacturer. RNA samples (40 µg) were DNase I (Thermo Scientific) treated in a 100 µl volume and diluted to 100 ng µl^{−1}. RNA samples were reverse transcribed and used as the template for Real-Time PCR with Express One-Step SYBR GreenER (Invitrogen) in a 20 µl total reaction volume. Real-Time PCR was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems) with the oligonucleotides (Sigma) ATATGTGGGTTCCTCTGG and TTGGTGGCTCTTAATCGTAA (ompC), GAAACCGGAT TACACCCGA and ACTCTCGGTATTTCAACCC (ompF) and TACCTGCTGCGGAGAGAAACGCAGAT (3′). Data were analysed by using the comparative Ct method where target gene transcription of each sample was normalized to the Ct of the waaY transcript.

Electrophoretic mobility shift assay (EMSA)

For preparation of recombinant OmpR–6×His S. Typhi genomic DNA was PCR-amplified using oligonucleotide primers 5′ CATGCCATGGaagagaattataagattctgg 3′ and 5′ CCGCTCGAGgtcttaggaacgcgcgtac 3′. The amplified DNA was cloned into pET28 vector into the NcoI and XhoI restriction sites giving rise to pTW1. One litre of E. coli BL21 pTW1 was cultured in Luria–Bertani containing 1 mM IPTG broth at 25°C to OD_{600} of 0.6. Cells were disrupted using a constant cell disruptor (Constant Systems), centrifuged at 23500 rcf and OmpR–6×His purified from the supernatant by affinity chromatography using nickel-resin chromatography. OmpR was phosphorylated with lithium potassium acetyl phosphate as previously described (Kenney et al., 1995). Double-stranded DNA probes were either PCR-amplified from S. Typhi genomic DNA using primers 5′ 6-FAM – AAC GGGATTTTACACAAACAG 3′ and 5′ 6-FAM – AGTC ATTATCATATCTTAAAATTGG 3′ (probe 1), or by annealing the oligonucleotides 5′ 6-FAM – TCAAAATAAGAATT CCTAATCGTATTTGAAATAATCTGTTACAAATTTAATTGTTT
GCACCTTTGGGGTTAAA 3’ and 5’ 6-FAM – TTAA CCCAAAGGTGCAAAACATTTAATTTGTAACAGATTATTT CAAATACGATAGAATTTCTTTTGGTA 3’. Probes with mutated putative binding motif were generated by overlap extension PCR using the oligonucleotide primers 5’ cat agaagattgacagtattcc 3’, 5’ caataaatgcgcagcattattcataatcagattagattaatttc 3’, 5’ ag tgtatcacccactacccagg 3’ and 5’ gaaataatgcgcagcatttaatgttgacacctttggg 3’, and subsequent amplification with 6-FAM labelled primers above. EMSA binding assay was performed in 10 mM Tris.HCl pH 7.2, 50 mM KCl, 5 mM MgCl₂, 1 mM EDTA, 1 mM DTT, 1 mg ml⁻¹ BSA, 0.001 mg of poly(dIdC) and 5% glycerol. 10 nM OmpR–6×His was incubated with various concentrations of 6-carboxyfluorescein (6-FAM)-labelled probe DNA shaking for 35 min at 30°C. Samples were separated on a 10% TBE polyacrylamide gel (Biorad) and 6-FAM-labelled nucleic acid imaged using a Typhoon 9200 (Amersham).

Animal experiments

In all mouse experiments female, 7–8 week-old C57BL/6 mice (Charles River) were inoculated orally by gavage with S. Typhimurium suspended in PBS pH 7.4. For mixed inoculum experiments in order to distinguish the wild-type strain from the mutant test strains, a cat (chloramphenicol acetyltransferase, chloramphenicol resistance gene) was inserted in the S. Typhimurium SL1344 chromosome in a position that has previously been described to have no effect on colonization of the murine host (Kingsley et al., 2003; Winter et al., 2010) (phoN locus, strain RAK113). Groups of five mice were inoculated orally with a 1:1 (log 10) ratio of approximately 1 × 10⁶ cfu of strain RAK113 and the test strain. When mice were moribund (less than 80% body weight compared with day of inoculation) or on day 5 post inoculation, mice were culled and cfu of each strain in homogenized mesenteric lymph nodes (MLN), caecum, ileum, spleen and liver was determined by serial dilution in PBS pH 7.4 and plating on LB agar containing cloramphenicol and LB agar containing kanamycin. Serial 10-fold dilutions were plated on LB + Cm or LB + Km agar, as appropriate, to determine cfu per organ. The ratio of wild-type (strain RAK113) to test strain was transformed to log₁₀ and to determine if these values were significantly different from the log₁₀ of the input ratio (input ratio log₁₀ = 0) was determined using the two-tailed Student’s t-test in the Prism 4 software version 4.0c (Graph Pad). Value for P < 0.05 was considered significantly different.

Minimal media growth assays

Salmonella Typhimurium SL1344 and isogenic mutant strains were grown overnight and washed three times in PBS and then plated onto M9 minimal media supplemented with L-histidine (for SL1344 growth) and 1% agar. The glucose carbon source was substituted with amino sugars. Wild-type controls were grown concomitantly on separate plates made from the same agar mix.

Ethics statement

All animal procedures were performed in accordance with the United Kingdom Home Office Inspectorate under the Animals (Scientific Procedures) Act 1986. The Wellcome Trust Sanger Institution Ethical Review Committee granted ethical approval for these procedures.

Acknowledgements

The work described here was funded by the Wellcome Trust through core funding for the Sanger Institute Pathogen Variation Group. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The authors have no conflicts of interest to declare.

References

Balbontin, R., Rowley, G., Pucciarelli, M.G., Lopez-Garrido, J., Wormstone, Y., Lucchini, S., et al. (2006) DNA adenine methylation regulates virulence gene expression in Salmonella enterica serovar Typhimurium. J Bacteriol 188: 8160–8168.

Bäumler, A.J. (1997) The record of horizontal gene transfer in Salmonella. Trends Microbiol 5: 318–322.

Bäumler, A.J., Gilde, A.J., Tolis, R.M., van der Velden, A.W.M., Ahmer, B.M.M., and Heffron, F. (1997) Contribution of horizontal gene transfer and deletion events to the development of distinctive patterns of fimbrial operons during evolution of Salmonella serotypes. J Bacteriol 179: 317–322.

Bertino, Y., Chaucheyras-Durand, F., Robbe-Masselot, C., Durand, A., Foye, A., Harel, J., et al. (2012) Carbohydrate utilization by enterohaemorrhagic Escherichia coli O157:H7 in bovine intestinal content. Environ Microbiol. doi: 10.1111/1462-2920.12019

Blattner, F., Plunkett, G., Bloch, C., Perna, N., Burland, V., Riley, M., et al. (1997) The complete genome sequence of Escherichia coli K-12. Science 277: 1453–1462.

Cameron, A.D., and Dorman, C.J. (2012) A fundamental regulatory mechanism operating through OmpR and DNA topology controls expression of Salmonella pathogenicity islands SPI-1 and SPI-2. PLoS Genet 8: e1002615.

Campbell, J.W., Morgan-Kiss, R.M., and Cronan, J.E. (2003) A new Escherichia coli metabolic competency: growth on fatty acids by a novel anaerobic beta-oxidation pathway. Mol Microbiol 47: 793–805.

Chang, D.E., Smalley, D.J., Tucker, D.L., Leatham, M., Norris, W.E., Stevenson, S.J., et al. (2004) Carbon nutrition of Escherichia coli in the mouse intestine. Proc Natl Acad Sci USA 101: 7427–7432.

Clarke, T.A., Mills, P.C., Poock, S.R., Butt, J.N., Cheesman, M.R., Cole, J.A., et al. (2008) Escherichia coli cytochrome c nitrite reductase NirA. Methods Enzymol 437: 63–77.

Cunningham, L., and Guest, J.R. (1998) Transcription and transcript processing in the sdhCDAB–sucABCD operon of Escherichia coli. Microbiology 144 (Pt 8): 2113–2123.

Feng, X., Oropeza, R., and Kenney, L.J. (2003) Dual regulation by phospho-OmpR of sarA/B gene expression in Salmonella pathogenicity island 2. Mol Microbiol 48: 1131–1143.

Forst, S.A., and Roberts, D.L. (1994) Signal transduction by the EnvZ–OmpR phosphotransfer system in bacteria. Res Microbiol 145: 363–373.

© 2012 Blackwell Publishing Ltd, Molecular Microbiology, 87, 526–538
Giraud, A., Arous, S., Paepe, M.D., Gaboriau-Routhiau, V., Bambou, J.C., Rakotobe, S., et al. (2008) Dissecting the genetic components of adaptation of *Escherichia coli* to the mouse gut. *PLoS Genet* 4: e2.

Golevbeva, Y.A., Sadik, A.Y., Ellermeier, J.R., and Slauch, J.M. (2012) Integrating global regulatory input into the *Salmonella* pathogenicity island 1 type III secretion system. *Genetics* 190: 79–90.

Groisman, E.A., and Ochman, H. (1997) How *Salmonella* became a pathogen. *Trends Microbiol* 5: 343–349.

Hapfelmeier, S., and Hardt, W.D. (2005) A mouse model for *Salmonella* enterica serovar Typhimurium-induced enterocolitis. *Trends Microbiol* 13: 497–503.

Hapfelmeier, S., Ehrbar, K., Stecher, B., Barthel, M., Kremer, M., and Hardt, W.D. (2004) Role of the *Salmonella* pathogenicity island 1 effector proteins SipA, SopB, SopE, and SopE2 in *Salmonella enterica* subspecies 1 serovar Typhimurium colitis in streptomycin-pretreated mice. * Infect Immun* 72: 795–809.

Harari, O., Park, S.Y., Huang, H., Groisman, E.A., and Zvir, I. (2010) Defining the plasticity of transcription factor binding sites by deconstructing DNA consensus sequences: the PhoP-binding sites among gamma/enterobacteria. *PLoS Comput Biol* 6: e1000862.

Harlocker, S.L., Bergstrom, L., and Inouye, M. (1995) Tandem binding of six OmpR proteins to the ompF upstream regulatory sequence of *Escherichia coli*. *J Biol Chem* 270: 26849–26856.

Head, C.G., Tardy, A., and Kenney, L.J. (1998) Relative binding affinities of OmpR and OmpR-phosphate at the ompF and ompC regulatory sites. *J Mol Biol* 281: 857–870.

Huang, K.J., Schieberl, J.L., and Igo, M.M. (1994) A distant upstream site involved in the negative regulation of the *Escherichia coli* ompF gene. *J Bacteriol* 176: 1309–1315.

Kelly, A., Goldberg, M.D., Carroll, R.K., Danino, V., Hinton, J.C., and Dorman, C.J. (2004) A global role for Fis in the transcriptional control of metabolism and type III secretion in *Salmonella enterica* serovar Typhimurium. *Microbiology* 150: 2037–2053.

Kenney, L.J., Bauer, M.D., and Silhavy, T.J. (1995) Phosphorylation-dependent conformational changes in OmpR, an osmoregulatory DNA-binding protein of *Escherichia coli*. *Proc Natl Acad Sci USA* 92: 8866–8870.

Kingsley, R.A., Humphries, A.D., Weening, E.H., Zoete, M.R.D., Winter, S., Papaconstantinopoulou, A., et al. (2003) Molecular and phenotypic analysis of the CS54 island of *Salmonella enterica* serotype Typhimurium: identification of intestinal colonization and persistence determinants. * Infect Immun* 71: 629–640.

Lowe, D.C., Savidge, T.C., Pickard, D., Eckmann, L., Kagnoff, M.F., Dougan, G., and Chatfield, S.N. (1999) Characterization of candidate live oral *Salmonella typhi* vaccine strains harboring defined mutations in aroA, aroC, and *htrA*. * Infect Immun* 67: 700–707.

Oropeza, R., and Calva, E. (2009) The cysteine 354 and 277 residues of *Salmonella enterica* serovar Typhi EnvZ are determinants of autophosphorylation and OmpR phosphorylation. *FEMS Microbiol Lett* 292: 282–290.

Oshima, T., Alba, H., Masuda, Y., Kanaya, S., Sugira, M., Wanner, B.L., et al. (2002) Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. *Mol Microbiol* 46: 281–291.

Parkhill, J., Dougan, G., James, K.D., Thomson, N.R., Pickard, D., Wain, J., et al. (2001) Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. *Nature* 413: 848–852.

Perkins, T.T., Kingsley, R.A., Fookes, M.C., Gardner, P.P., James, K.D., Yu, L., et al. (2009) A strand-specific RNA-Seq analysis of the transcriptome of the typhoid bacillus *Salmonella typhi*. *PLoS Genet* 5: e1000569.

Pfeiffer, V., Sittka, A., Tomer, R., Tedin, K., Brinkmann, V., and Vogel, J. (2007) A small non-coding RNA of the invasion gene island (SPI-1) represses outer membrane protein synthesis from the *Salmonella* core genome. *Mol Microbiol* 66: 1174–1191.

Pickard, D., Li, J., Roberts, M., Maskell, D., Hone, D., Levine, M., et al. (1994) Characterization of defined ompR mutants of *Salmonella typhi*. ompR is involved in the regulation of Vi polysaccharide expression. *Infect Immun* 62: 3984–3993.

Plumbidge, J., and Virr, E. (1999) Convergent pathways for utilization of the amino sugars N-acetylglucosamine, N-acetylmannosamine, and N-acetylneuraminic acid by *Escherichia coli*. *J Bacteriol* 181: 47–54.

Popoff, M.Y., Bockemühl, J., and Gheebling, L.L. (2004) Supplement 2002 (No. 46) to the Kauffmann-White scheme. *Res Microbiol* 155: 568–570.

Rhee, J.E., Sheng, W., Morgan, L.K., Nolet, R., Liao, X., and Kenney, L.J. (2008) Amino acids important for DNA recognition by the response regulator OmpR. *J Biol Chem* 283: 8664–8677.

Rowe, J.J., Ubbink-Kok, T., Molenaar, D., Konings, W.N., and Driessen, A.J. (1994) NarK is a nitrate-extrusion system involved in anaerobic nitrate respiration by *Escherichia coli*. *Mol Microbiol* 12: 579–586.

Rutherford, K., Parkhill, J., Crook, J., Horsnell, T., Rice, P., Rajandream, M.A., and Barrell, B. (2000) Artemis: sequence visualization and annotation. *Bioinformatics* 16: 944–945.

Santos, R.L., Zhang, S., Tsolis, R.M., Kingsley, R.A., Adams, L.G., and Baumler, A.J. (2001) Animal models of *Salmonella* infections: enteritis versus typhoid fever. *Microbes Infect* 3: 1335–1344.

Severi, E., Randle, G., Kivlin, P., Whitfield, K., Young, R., Moxon, R., et al. (2005) Sialic acid transport in *Haemophilus influenzae* is essential for lipopolysaccharide sialylation and serum resistance and is dependent on a novel tripartite ATP-independent periplasmic transporter. *Mol Microbiol* 58: 1173–1185.

Severi, E., Hood, D.W., and Thomas, G.H. (2007) Sialic acid utilization by bacterial pathogens. *Microbiology* 153: 2817–2822.

Severi, E., Muller, A., Potts, J.R., Leech, A., Williamson, D., Wilson, K.S., and Thomas, G.H. (2008) Sialic acid mutarotation is catalyzed by the *Escherichia coli* beta-propeller protein YfHT. *J Biol Chem* 283: 4841–4849.

Severi, E., Hosie, A.H., Hawkhead, J.A., and Thomas, G.H. (2010) Characterization of a novel sialic acid transporter of the sodium solute symporter (SSS) family and in vivo comparison with known bacterial sialic acid transporters. *FEMS Microbiol Lett* 304: 47–54.
Sinha, S., and Tompa, M. (2003) YMF: a program for discovery of novel transcription factor binding sites by statistical overrepresentation. *Nucleic Acids Res* **31**: 3586–3588.

Stecher, B., Robbiani, R., Walker, A.W., Westendorf, A.M., Barthel, M., Kremer, M., *et al.* (2007) *Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol* **5**: 2177–2189.

Stecher, B., Barthel, M., Schlumberger, M.C., Haberli, L., Rabsch, W., Kremer, M., and Hardt, W.D. (2008) Motility allows *S. Typhimurium* to benefit from the mucosal defence. *Cell Microbiol* **10**: 1166–1180.

Thiennimitr, P., Winter, S.E., Winter, M.G., Xavier, M.N., Tolstikov, V., Huseby, D.L., *et al.* (2011) Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. *Proc Natl Acad Sci USA* **108**: 17480–17485.

Tsolis, R.M., Kingsley, R.A., Townsend, S.M., Ficht, T.A., Adams, L.G., and Baumler, A.J. (1999) Of mice, calves, and men. Comparison of the mouse typhoid model with other *Salmonella* infections. *Adv Exp Med Biol* **473**: 261–274.

Turner, A.K., Nair, S., and Wain, J. (2006) The acquisition of full fluoroquinolone resistance in *Salmonella* Typhi by accumulation of point mutations in the topoisomerase targets. *J Antimicrob Chemother* **58**: 733–740.

Winter, S.E., Thiennimitr, P., Winter, M.G., Butler, B.P., Huseby, D.L., Crawford, R.W., *et al.* (2010) Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* **467**: 426–429.

**Supporting information**

Additional supporting information may be found in the online version of this article.
Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Perkins, TT; Davies, MR; Klemm, EJ; Rowley, G; Wileman, T; James, K; Keane, T; Maskell, D; Hinton, JCD; Dougan, G; Kingsley, RA

Title:
ChIP-seq and transcriptome analysis of the OmpR regulon of Salmonella enterica serovars Typhi and Typhimurium reveals accessory genes implicated in host colonization

Date:
2013-02-01

Citation:
Perkins, T. T., Davies, M. R., Klemm, E. J., Rowley, G., Wileman, T., James, K., Keane, T., Maskell, D., Hinton, J. C. D., Dougan, G. & Kingsley, R. A. (2013). ChIP-seq and transcriptome analysis of the OmpR regulon of Salmonella enterica serovars Typhi and Typhimurium reveals accessory genes implicated in host colonization. MOLECULAR MICROBIOLOGY, 87 (3), pp.526-538. https://doi.org/10.1111/mmi.12111.

Persistent Link:
http://hdl.handle.net/11343/260634

File Description:
Published version

License:
CC BY