Supplemental Information

An Infection-Relevant Transcriptomic Compendium for *Salmonella enterica* Serovar Typhimurium
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Figure S1
Figure S1 (related to Figure 2): Expression analysis of S. Typhimurium genes, Relative RNA-seq read distribution and 5’RACE of a secondary TSS of *hilD* (a) and the primary TSS of *stpA*. (A) Percentage of expressed genes in 22 environmental conditions and the RNA pool. Expression was determined based on a cut-off of TPM >10. (B) Functional categorization of genes and whether they are expressed or not. Most genes that are not expressed belong to the groups of “surface structures”, “cell motility and secretion” and “carbohydrate transport and metabolism”. (C) The bar chart shows categorization and the relative distribution of reads that map only into a single location on the chromosome (“uniquely mapped reads”, Dataset 1) within the cDNA libraries. SRP: Signal recognition particle. (D) Agarose gels (left) of RT-PCR products of tobacco acid pyrophosphate treated (TAP; T+) and mock-treated (T−) and of a control PCR reaction with genomic DNA as template (ctr.). The arrowheads mark the enriched band in TAP-treated samples, indicating the cDNA of the respective primary RNA species. A DNA size marker is shown on the left [M, sizes in base pairs (bp)]. Sequence up- and down-stream of the TSS are shown (right) with likely -10 and -35 regions marked with red boxes. The initiating nucleotide is labeled in black and the number refers to the distance to the respective translational starts.
Figure S2
Figure S2 (related to Figure 3): Mapped sequence reads visualized in IGB and figures show SPI2 (A) and SPI3 (B). The scale is 0-100 normalized reads for every sample. Protein coding gene names are labeled in black, sRNA gene names in blue. Transcriptional start sites are marked by arrows.
Figure S3
Figure S3 (related to Figure 3): Mapped sequence reads visualized in IGB and figures show SPI4-5 (A) and SPI11 (B). The scale is 0-100 normalized reads for every sample. Protein coding gene names are labeled in black, sRNA gene names in blue. Transcriptional start sites are marked by arrows.
Figure S4
Figure S4 (related to Figure 3): Mapped sequence reads visualized in IGB and figures SPI12 (A) and SPI16 (B). The scale is 0-100 normalized reads for every sample. Protein coding gene names are labeled in black, sRNA gene names in blue. Transcriptional start sites are marked by arrows.
Figure S5: Absolute gene expression for all SPIs (related to Figure 4). Heatmaps are based on TPM expression values (Dataset 2). Coding gene names are labeled in black, sRNA gene names in blue. SPI3-11 is shown in (A), SPI12-16 in (B).
Figure S6
Figure S6: Relative gene expression for all SPIs (related to Figure 5). Coding gene names are labeled in black, sRNA gene names in blue. Each box shows a separate comparison. Comparators are labeled in bold (EEP, MEP, Anaerobic growth, InSPI2 and NonSPI2). SPIs 3-11 are shown (A), SPIs 12-16 are shown in (B).
**Figure S7 (related to Figure 7):** Northern blots and mapped sequence reads of new sRNAs. 5-10 μg of total RNA was loaded onto urea-polyacrylamide gels. Every box shows a Northern blot and mapped sequence reads of a new sRNA in conditions where its expression is low or high. The arrowheads show the most prominent band. 5S rRNA served as a loading control. The estimated length based on the RNA-seq data is given in brackets.

**Table S1: Oligonucleotides used in this study:**

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EXTENDED EXPERIMENTAL PROCEDURES:

**Detailed growth conditions.** Bacteria were grown to early exponential growth phase (EEP; OD$_{600}$ 0.1), mid exponential growth phase (MEP; OD$_{600}$ 0.3), late exponential growth phase (LEP; OD$_{600}$ 1.0), early stationary phase (ESP, OD$_{600}$ 2.0) and late stationary phase (LSP, OD$_{600}$ 2.0 + 6h). Environmental shocks in L-broth were performed on MEP-grown bacteria as follows: Osmotic shock (NaCl shock), addition of NaCl to a final concentration of 0.3 M and continued growth for 10 min; iron limitation (LowFe$^{2+}$ shock), addition of 2,2'-dipyridyl to a final concentration of 0.2 mM and continued growth for 10 min (McHugh et al., 2003); cold shock, flasks were transferred to a second water bath incubator maintained at 15°C for 15 min at 220 rpm; mild acidic stress (pH5.8 shock) and strong acidic shock (pH3 shock), cells were pelleted for 5 min at 3230 x g, resuspended in pre-warmed (37°C) L-broth (adjusted to pH5.8 or pH3 with HCl) and grown for 10 min; anaerobic shock, 15ml culture were transferred into a pre-warmed (37°C) 15 ml centrifuge tube, screwed tight and incubated without agitation for 30 min; bile salt stress (Bile shock), addition of ox bile (sodium choleate) to a final concentration of 3% (w/v) and continued growth for 10 min (Prouty and Gunn, 2000). For growth at low temperature (25°C) an overnight culture of 5 ml L-broth grown at 37°C was diluted 1:1000 in a 250 ml flask containing 25 ml L-broth and incubated in a water bath incubator at 25°C and 220 rpm and RNA was isolated at OD$_{600}$ 0.3. For temperature shifts (Temp10, Temp20), cells were grown at 25°C (water-bath, 220 rpm) until OD$_{600}$ of 0.3 and then transferred to 37°C (water-bath, 220 rpm) and RNA was isolated after 10 and 20 min. For growth in anaerobic conditions (anaerobic growth), a 50 ml centrifuge tube was filled with L-broth and inoculated with 50 µl (1:1000) of an overnight culture grown in 5 ml L-broth, screwed tight, incubated without agitation at 37°C and RNA was isolated from cells grown to OD$_{600}$ 0.3. For oxygen shock, 25 ml of cells grown anaerobically to OD$_{600}$ 0.3, as described above, were transferred to a 250 ml baffled flask, agitated in a water bath incubator at 37°C and 250 rpm, and RNA was isolated after 15 min. For growth in variants of phosphate carbon nitrogen (PCN) minimal medium (Löber et al., 2006), cells were grown over night in 5 ml L-broth and the next morning 1 ml culture was washed twice with, and then diluted 1:1000 in 250 ml flasks in 25 ml in either SPI2-inducing PCN (InSPI2; pH5.8, 0.4 mM Pi), SPI2-non-inducing PCN (NonSPI2; pH7.4, 25 mM Pi) or SPI2-inducing PCN (LowMg$^{2+}$; pH5.8, 0.4 mM Pi) containing low levels (10 µM) of magnesium sulfate. RNA was isolated at OD$_{600}$ of 0.3. The shocks conducted in PCN (InSPI2) were done at OD$_{600}$ 0.3: addition of peroxide (Peroxide shock (InSPI2)) to a final concentration of 1 mM for 12 min (Wright et al., 2009); addition of nitric oxide (Nitric oxide shock (InSPI2)) donor Spermine NONOate to a final concentration of 250 µM for 20 min (Bourret et al., 2008).
Small RNA identification and Hfq-enrichment factors of sRNAs: The criteria for the identification of new small RNA candidates included the presence of one or more of the following characteristics, i.e. a small (<500 nucleotides) transcript followed by a predicted ρ-independent transcriptional terminator and/or binding to Hfq according to published Hfq-coIP-seq data (Chao et al., 2012; Sittka et al., 2008). Hfq-enrichment factors were calculated as in Chao et al. (2012) from the Hfq-coIP-seq experiments carried out by Chao et al. (GEO database accession number GSE38884) and Sittka et al. (Chao et al., 2012; Sittka et al., 2008). A full sRNA table is presented in Dataset 4 (Altuvia et al., 1997; Argaman et al., 2001; Balbontin et al., 2008; Bossi and Figueroa-Bossi, 2007; Boysen et al., 2010; Chao et al., 2012; Corcoran et al., 2012; De Lay and Gottesman, 2009; Douchin et al., 2006; Durand and Storz, 2010; Ellermeier and Slauch, 2008; Figueroa-Bossi et al., 2009; Fortune et al., 2006; Fozo et al., 2008; Fröhlich et al., 2012; Guillier and Gottesman, 2008; Hartog et al., 2008; Hershberg et al., 2003; Holmqvist et al., 2010; Johansen et al., 2008; Majdalani et al., 2001; Mizuno et al., 1984; Moller et al., 2002; Moon and Gottesman, 2009; Nechooshtan et al., 2009; Padalon-Brauch et al., 2008; Papenfort et al., 2008; Papenfort et al., 2012; Papenfort et al., 2013; Pfeiffer et al., 2009; Pfeiffer et al., 2007; Ramachandran et al., 2012; Rivas et al., 2001; Rudd, 1999; Santiviago et al., 2009; Sharma et al., 2007; Sittka et al., 2008; Sittka et al., 2009; Sridhar et al., 2010; Urban et al., 2007; Urban and Vogel, 2008; Vogel et al., 2003; Wadler and Vanderpool, 2009; Wassarman et al., 2001; Zhang et al., 2003).

Northern blot analysis and rapid amplification of cDNA ends (5′RACE): Primers used for PCRs to generate DNA templates for in vitro transcription of DIG-labelled riboprobes using T7 RNA polymerase and primers used in 5′RACE are listed in Table S1. The riboprobes were designed to usually cover the full length of the sRNA with a minimum length of ~50 nt. For detection of new small RNA candidates, 5 or 10 µg of total RNA was loaded on polyarcrylamide-urea gels (7% polyacrylamide, 8.3 M urea in 1 x Tris-borate EDTA (TBE) buffer) and non-radioactive Northern blots were conducted using the DIG Northern blot starter kit (Roche) according to the manufacturer’s manual. Bands were visualised using an ImageQuant LAS4000 imager (GE healthcare). 5′RACE experiments to confirm TSS of stpA and hilD were carried out as described earlier in Wright et al (Vogel et al., 2003).

SUPPLEMENTAL REFERENCES:


