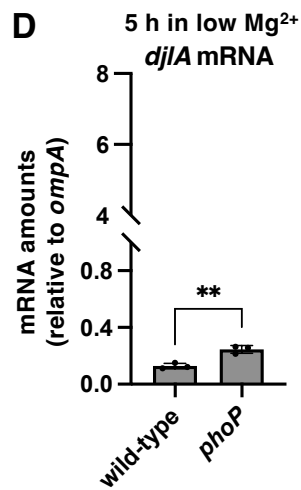
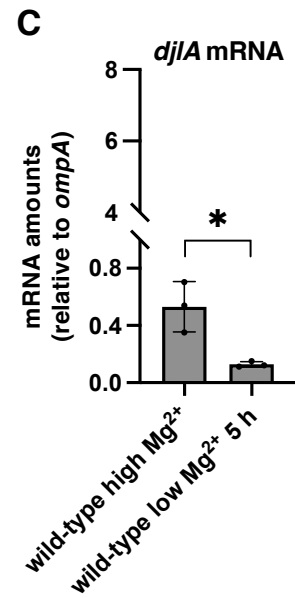
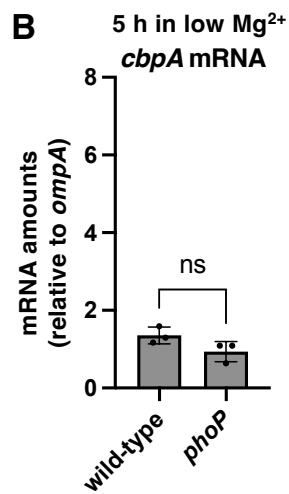
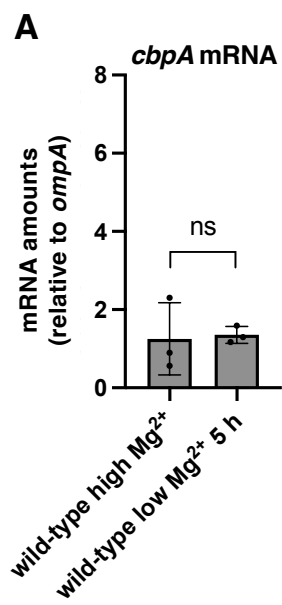
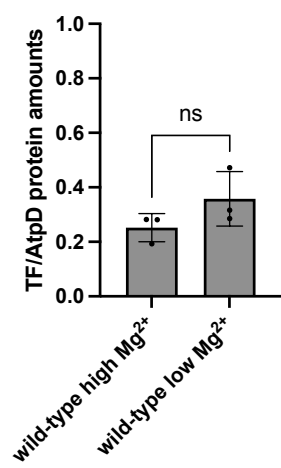
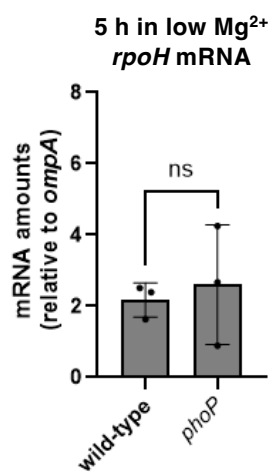


Supplemental Material for

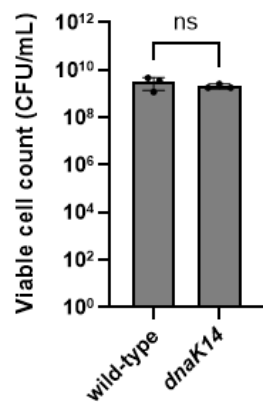
**Infection-relevant conditions dictate differential versus coordinate
expression of *Salmonella* chaperones and cochaperones**



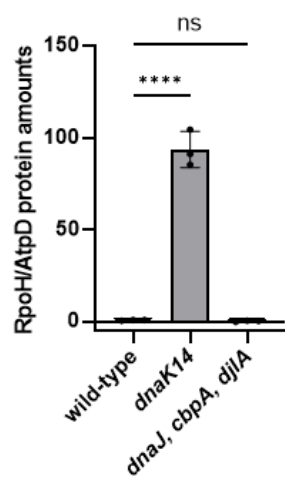




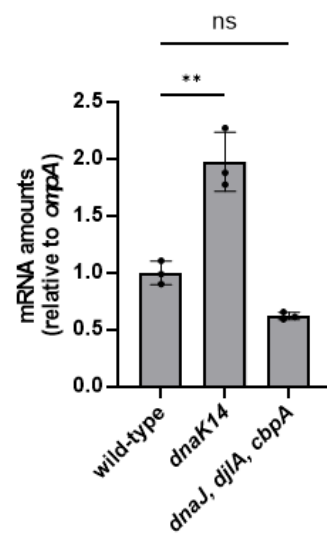
A 5 h in high Mg^{2+}



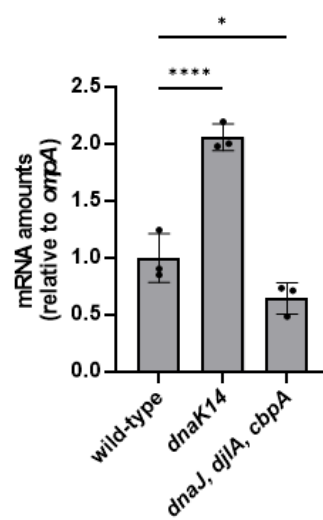
B 4.5 h in high Mg^{2+}



C 5 h in low Mg^{2+}
creA mRNA



D 4.5 h in high Mg^{2+}
creA mRNA



Supporting Figure Legends

Supporting Figure S1: The mRNA abundance of J-protein cochaperone-encoding genes does not increase when *S. Typhimurium* experiences cytoplasmic Mg^{2+} starvation.

(A) mRNA abundance of the *cbpA* gene relative to the constitutive *ompA* control in wild-type (14028s) *S. Typhimurium* following 4.5 h of growth in high (10 mM) Mg^{2+} or 5 h of growth in low (10 μ M) Mg^{2+} .

(B) mRNA abundance of the *cbpA* gene relative to the constitutive *ompA* control in wild-type (14028s) and *phoP* (MS7953s) *S. Typhimurium* following 5 h of growth in low (10 μ M) Mg^{2+} .

(C) mRNA abundance of the *djlA* gene relative to the constitutive *ompA* control in wild-type (14028s) *S. Typhimurium* following 4.5 h of growth in high (10 mM) Mg^{2+} or 5 h of growth in low (10 μ M) Mg^{2+} .

(D) mRNA abundance of the *djlA* gene relative to the constitutive *ompA* control in wild-type (14028s) and *phoP* (MS7953s) *S. Typhimurium* following 5 h of growth in low (10 μ M) Mg^{2+} .

Data in (A-D) represent mean \pm SD of three independent biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test comparing the bracketed sample groups (* $p < 0.05$, ** $p < 0.01$, ns = not significant).

Supporting Figure S2: Trigger Factor protein amounts are similar during growth in high Mg^{2+} and low Mg^{2+} .

Protein amounts of TF relative to the AtpD loading control determined by Western blot in wild-type (14028s) *S. Typhimurium* following 4.5 h of growth in high (10 mM) Mg^{2+} or 5 h of growth in low (10 μM) Mg^{2+} .

Data represent mean \pm SD of three independent biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test comparing the bracketed sample groups (ns = not significant).

Supporting Figure S3: Inactivation of the *phoP* gene does not alter *rpoH* mRNA amounts.

mRNA abundance of the *rpoH* gene relative to the constitutive *ompA* control in wild-type (14028s) and *phoP* (MS7953s) *S. Typhimurium* following 5 h of growth in low (10 μM) Mg^{2+} .

Data represent mean \pm SD of three independent biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test comparing the bracketed sample groups (ns = not significant).

Supporting Figure 4: C-terminal domain of DnaK is required to decrease RpoH amounts and activity during growth in high Mg^{2+} .

(A) Survival of wild-type (14028s) and *dnaK14* (CC186) *S. Typhimurium* following 5 h of growth in low (10 μM) Mg^{2+} .

(B) Protein amounts of RpoH relative to the AtpD loading control determined by Western blot in wild-type (14028s), *dnaK14* (CC186), and *dnaJ cbpA djlA* (CC656) *S. Typhimurium* following 4.5 h of growth in high (10 mM) Mg^{2+} .

(C) mRNA abundance of the *creA* gene relative to that of the constitutive *ompA* control in wild-type (14028s), *dnaK14* (CC186), and *dnaJ cbpA djlA* (CC656) *S. Typhimurium* following 5 h of growth in low (10 μ M) Mg^{2+} .

(D) mRNA abundance of the *creA* gene relative to that of the constitutive *ompA* control in wild-type (14028s), *dnaK14* (CC186), and *dnaJ cbpA djlA* (CC656) *S. Typhimurium* following 4.5 h of growth in high (10 mM) Mg^{2+} .

Data in (A-C) represent mean \pm SD of three independent biological replicates. Statistical analysis was performed using two-tailed Student's *t*-test comparing the bracketed sample groups (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, ns = not significant).

Table S1. Strains and plasmids used in this study

| Strains | Relevant characteristics | Source |
|---|---|---------------------------|
| <i>Salmonella enterica</i> serovar Typhimurium | | |
| 14028s | wild-type | (Fields et al., 1986) |
| MS7953s | <i>phoP</i> ::Tn10 | (Fields et al., 1986) |
| EL1 | <i>mgtC</i> :: <i>kan</i> | (Lee et al., 2013) |
| CC186 | <i>dnaK14</i> ::Tn10 <i>dCm</i> (insertion at nucleotide position 1691) | (Chan and Groisman, 2024) |
| CC656 | <i>dnaJ</i> , <i>cbpA</i> , <i>djlA</i> :: <i>kan</i> | (Chan and Groisman, 2024) |
| | | |
| <i>Escherichia coli</i> | | |
| DH5 α | Host strain used for generation and propagation of plasmid constructs | (Hanahan, 1985) |
| | | |
| Plasmids | | |
| pUHE-21-2-lacI ^q | rep _{pMB1} lacI ^q Amp ^R vector control | (Soncini et al., 1995) |
| pAtpAGD | pUHE-21-2-lacI ^q -AtpAGD | (Pontes et al., 2016) |

Table S2. Oligonucleotides used in this study

| Oligo name | Sequence (5' to 3') | Purpose | Source |
|------------|--------------------------------------|--|------------------------|
| 17717 | TTA TTG GTA TCG ACC TGG GTA CTA C | <i>dnaK</i> mRNA qRT-PCR quantification | This work |
| 17718 | CAG AGT TTC ACC ATC CTG GGT ATA A | <i>dnaK</i> mRNA qRT-PCR quantification | This work |
| 16274 | AAGCCTACGAAGTGCT GACC | <i>dnaJ</i> mRNA qRT-PCR quantification | This work |
| 16275 | ATTAAAGCCGCCGCCA AATC | <i>dnaJ</i> mRNA qRT-PCR quantification | This work |
| 18914 | CGCCATTATGGGCGTG AAACC | <i>cbpA</i> mRNA qRT-PCR quantification | This work |
| 18915 | CGGGTTCTTTGCTGACA TCTGG | <i>cbpA</i> mRNA qRT-PCR quantification | This work |
| 18916 | gataattggcgtcgccgtagc | <i>djlA</i> mRNA qRT-PCR quantification | This work |
| 18917 | ccatttttcggctgcgggc | <i>djlA</i> mRNA qRT-PCR quantification | This work |
| 15054 | gggctgggtctcagtaccatga | <i>ompA</i> mRNA qRT-PCR quantification | (Yeom et al., 2017) |
| 15055 | tcatgagtcgggcatca | <i>ompA</i> mRNA qRT-PCR quantification | (Yeom et al., 2017) |
| 19251 | gacgacgttgctgacgctg | <i>dnaKJ</i> intergenic region qRT-PCR quantification | This work |
| 19252 | ccgctgttttgaaacgcc | <i>dnaKJ</i> intergenic region qRT-PCR quantification | This work |
| 19253 | ggaaatcgctcagcagcaac | <i>dnaK</i> 3' end qRT-PCR quantification | This work |
| 19254 | cttcttcaaactcagcgtagc | <i>dnaK</i> 3' end qRT-PCR quantification | This work |
| 19255 | ggcgaaaagagattactacgagatt tag | <i>dnaJ</i> 5' end qRT-PCR quantification | This work |

| | | | |
|-------|--|---|-----------|
| 19256 | cggatgatatttcatggccagg | <i>dnaJ</i> 5' end qRT-PCR quantification | This work |
| 17697 | TAA AAT TCG GTA ACG ACG CTC GTG T | <i>groEL</i> mRNA qRT-PCR quantification | This work |
| 17698 | ATA GTC GGC GCA CCG AAA GAT TTA T | <i>groEL</i> mRNA qRT-PCR quantification | This work |
| 18739 | GTA CTT TTC TCA ATT TTG TTG CTG CTG | <i>creA</i> mRNA qRT-PCR quantification | This work |
| 18740 | CAC CAC GAT TTT ATG GTC AGG ACC | <i>creA</i> mRNA qRT-PCR quantification | This work |