

Emergence of new sRNAs in enteric bacteria is associated with low expression and rapid evolution

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Crosslink-seq. (a) AMT crosslinked EcsR2 to mRNAs in the presence of UV (365 nm). (b) EcsR2 along with bound mRNAs were purified using affinity selection, and the mRNAs were uncrosslinked using UV (254 nm). (c) RNA-seq was utilized to identify mRNAs that were enriched in the strain expressing EcsR2 (Test) in comparison to the strain without EcsR2 (Control).

Figure S2. Predicted EcsR2-AnsB interaction. EcsR2-AnsB binding site as predicted by IntaRNA (Wright et al. 2014). Nucleotide positions on EcsR2 are based on taking its transcription start site (TSS) as +1. Nucleotide positions on AnsB are in relation to its translation start site (ATG) considered as +1. The TSS of AnsB is at -62 (Jennings and Beacham 1990).

Figure S3. Predicted evolution of sRNA secondary structure. The unstructured putative mRNA-binding region (top) and the intrinsic terminator (bottom) seem to have evolved in EcsR2. RNA secondary structure and free energy were predicted using ViennaRNA web service.

Figure S4. EcsR2 expression. EcsR2 was measured using semi-quantitative PCR (20 cycles). 16S rRNA gene was used as control.

SUPPLEMENTAL REFERENCES

Jennings MP, Beacham IR. 1990. Analysis of the *Escherichia coli* gene encoding L-asparaginase II, ansB, and its regulation by cyclic AMP receptor and FNR proteins. J Bacteriol. 172:1491-1498.

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Wright PR, Georg J, Mann M, Sorescu DA, Richter AS, Lott S, Kleinkauf R, Hess WR, Backofen R. 2014. CopraRNA and IntaRNA: predicting small RNA targets, networks and interaction domains. Nucleic Acids Res. 42:W119-W123.

Table S1. Age of *S. enterica* sRNAs correlates with expression during growth in 22 ‘infection-relevant’ conditions described in Kroger et al. 2013.

| Growth condition name | Growth description | Kruskal-Wallis test | | Young vs. Middle | | Middle vs. Old | | Young vs. Old | |
|----------------------------------|--|---------------------|--------|------------------|-------|----------------|-------|---------------|--------|
| | | Chi-Square | p | Z | p | Z | p | Z | p |
| EEP | Lennox broth OD600 0.1 | 22.35 | <0.001 | 2.36 | 0.018 | 2.66 | 0.008 | 4.61 | <0.001 |
| MEP | Lennox broth OD600 0.3 | 23.97 | <0.001 | 2.53 | 0.011 | 2.67 | 0.008 | 4.75 | <0.001 |
| LEP | Lennox broth OD600 1.0 | 15.94 | <0.001 | 1.8 | 0.071 | 2.42 | 0.016 | 3.94 | <0.001 |
| ESP average (Biol. Rep. 1 and 2) | Lennox broth OD600 2.0 | 18.52 | <0.001 | 1.88 | 0.061 | 2.67 | 0.008 | 4.35 | <0.001 |
| LSP average (Biol. Rep. 1 and 2) | Lennox broth OD600 2.0 + 6 hrs | 13.56 | 0.001 | 1.78 | 0.075 | 2.13 | 0.033 | 3.6 | <0.001 |
| 25°C | Lennox broth OD600 0.3 at 25°C | 24.38 | <0.001 | 2.36 | 0.018 | 2.87 | 0.004 | 4.84 | <0.001 |
| Cold shock (15°C) | Lennox broth OD600 0.3 + 15°C for 10 min | 27.32 | <0.001 | 2.44 | 0.015 | 3.1 | 0.002 | 5.14 | <0.001 |
| pH3 shock | Lennox broth OD600 0.3 + pH 3.0 for 10 min | 24.64 | <0.001 | 2.09 | 0.037 | 3.14 | 0.002 | 4.92 | <0.001 |
| pH5.8 shock | Lennox broth OD600 0.3 + pH 5.8 for 10 min | 21.6 | <0.001 | 2.45 | 0.014 | 2.48 | 0.131 | 4.5 | <0.001 |

| | | | | | | | | | |
|-------------------------------------|--|-------|--------|------|-------|------|--------|------|--------|
| NaCl shock | Lennox broth OD600 0.3 + 0.3 M NaCl for 10 min | 20.52 | <0.001 | 2.2 | 0.028 | 2.61 | 0.009 | 4.43 | <0.001 |
| Bile shock | Lennox broth OD600 0.3 + 3% bile for 10 min | 26.64 | <0.001 | 1.64 | 0.101 | 3.69 | <0.001 | 5.16 | <0.001 |
| LowFe2+ shock | Lennox broth OD600 0.3 + 0.2 mM 2,2'-dipyridyl for 10 min | 27.86 | <0.001 | 2.7 | 0.007 | 2.9 | 0.004 | 5.13 | <0.001 |
| Anaerobic shock | Lennox broth OD600 0.3 + growth in filled Falcon tube without agitation at 37°C for 30 min | 25.27 | <0.001 | 1.81 | 0.071 | 3.43 | <0.001 | 5.02 | <0.001 |
| Anaerobic growth | Static growth in Lennox broth to OD600 0.3 in a filled Falcom tube | 21.56 | <0.001 | 1.88 | 0.06 | 3 | 0.003 | 4.61 | <0.001 |
| Aerobic shock | Static growth in Lennox broth to OD600 0.3 in a filled Falcom tube + aerobic growth for 15 min | 18.1 | <0.001 | 2.35 | 0.019 | 2.17 | 0.03 | 4.08 | <0.001 |
| InSPI2 average (Biol. Rep. 1 and 2) | PCN medium (pH 5.8, 0.4 mM Pi) to OD600 0.3 | 14.36 | 0.001 | 2.17 | 0.03 | 1.85 | 0.064 | 3.61 | <0.001 |
| InSPI2 LowMg2+ | InSPI2 with 10 uM MgSO4 | 14.65 | 0.001 | 2.01 | 0.045 | 2.06 | 0.04 | 3.71 | <0.001 |
| Peroxide shock (InSPI2) | InSPI2 + 1 mM H2O2 for 12 min | 15.75 | <0.001 | 1.76 | 0.078 | 2.43 | 0.015 | 3.92 | <0.001 |

| | | | | | | | | | |
|-----------------------------|--|-------|--------|------|-------|------|--------|------|--------|
| Nitric oxide shock (InSPI2) | InSPI2 + 250 uM Spermine NONOate for 20 min | 18.67 | <0.001 | 2.03 | 0.042 | 2.54 | 0.011 | 4.24 | <0.001 |
| NonSPI2 | PCN medium (pH 7.4, 25 mM Pi) to OD600 0.3 | 16.11 | <0.001 | 2.12 | 0.034 | 2.14 | 0.032 | 3.88 | <0.001 |
| Temp10 | Lennox broth OD600 0.3 at 25°C + 37°C for 10 min | 24.73 | <0.001 | 2.32 | 0.021 | 2.95 | 0.003 | 4.89 | <0.001 |
| Temp20 | Lennox broth OD600 0.3 at 25°C + 37°C for 20 min | 19.68 | <0.001 | 2.03 | 0.043 | 2.67 | 0.008 | 4.37 | <0.001 |
| RNA pool | RNA from all 22 conditions pooled | 26.24 | <0.001 | 2.07 | 0.038 | 3.31 | <0.001 | 5.09 | <0.001 |

Permutation ANOVA

Age groups: $F(df=2,2770) = 153.5051$, p-value < 0.001

Growth conditions: $F(df=21,2770) = 0.7226$, p-value = 0.818

Post hoc Permutation T test on Age groups (FDR corrected):

Young vs. Middle: p-value = 0.052

Middle vs. Old: p-value = 0.003

Young vs. Old: p-value = 0.003

Table S2. *E. coli* genes significantly down-regulated by EcsR2 (RNA-seq).

| Gene | Fold Change | <i>p</i> -value |
|-------------|-------------|-----------------|
| <i>tdcA</i> | -18.66 | 4.26557E-08 |
| <i>ansB</i> | -16.12 | 1.71424E-07 |
| <i>yjdK</i> | -17.11 | 2.4002E-07 |
| <i>ynfK</i> | -14.21 | 2.4002E-07 |
| <i>yhbU</i> | -13.33 | 1.33517E-06 |
| <i>yehH</i> | -11.62 | 1.33517E-06 |
| <i>bssR</i> | -16.07 | 2.21521E-06 |
| <i>garP</i> | -19.94 | 2.38258E-06 |
| <i>garD</i> | -11.29 | 1.00801E-05 |
| <i>galS</i> | -9.31 | 1.55718E-05 |
| <i>ysaA</i> | -9.91 | 2.05315E-05 |
| <i>focA</i> | -8.40 | 2.39434E-05 |
| <i>yijM</i> | -7.08 | 0.00033494 |
| <i>gntK</i> | -6.72 | 0.000385247 |
| <i>chiP</i> | -11.47 | 0.001616576 |
| <i>melR</i> | -6.17 | 0.001896452 |
| <i>narG</i> | -10.67 | 0.001972134 |
| <i>abrB</i> | -5.73 | 0.003638417 |
| <i>yahN</i> | -6.49 | 0.003681708 |
| <i>araF</i> | -4.78 | 0.007342921 |
| <i>narK</i> | -19.24 | 0.017566139 |
| <i>ydjX</i> | -5.06 | 0.017566139 |
| <i>nirB</i> | -17.59 | 0.030360344 |
| <i>bsmA</i> | -15.17 | 0.033087754 |
| <i>raiA</i> | -3.93 | 0.037528124 |
| <i>feaR</i> | -4.08 | 0.049297294 |

Table S3. *E. coli* genes significantly enriched in Crosslink-seq.

| Gene | Fold Change | <i>p</i> -value |
|-------------|-------------|-----------------|
| <i>hslU</i> | 4.0 | 0.0001834 |
| <i>yedD</i> | 4.2 | 0.0004650 |
| <i>frdA</i> | 3.6 | 0.0000990 |
| <i>ansB</i> | 3.4 | 0.0000337 |
| <i>ygiB</i> | 3.5 | 0.0001740 |
| <i>adhE</i> | 3.2 | 0.0000007 |
| <i>gnd</i> | 3.2 | 0.0000000 |
| <i>glyA</i> | 2.7 | 0.0002994 |
| <i>ybaB</i> | 2.7 | 0.0000001 |

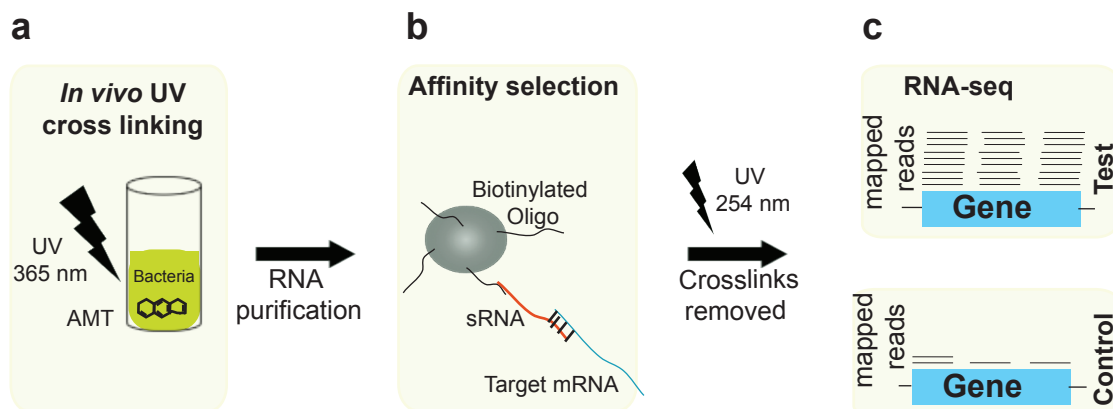


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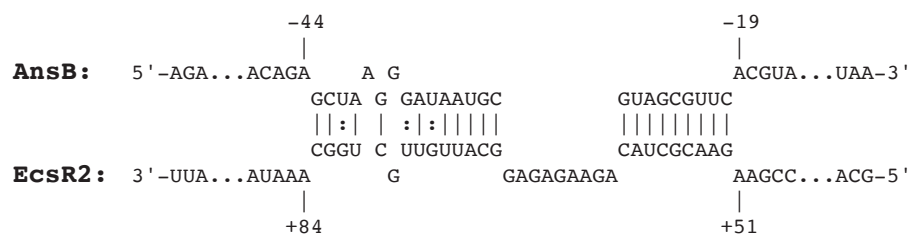


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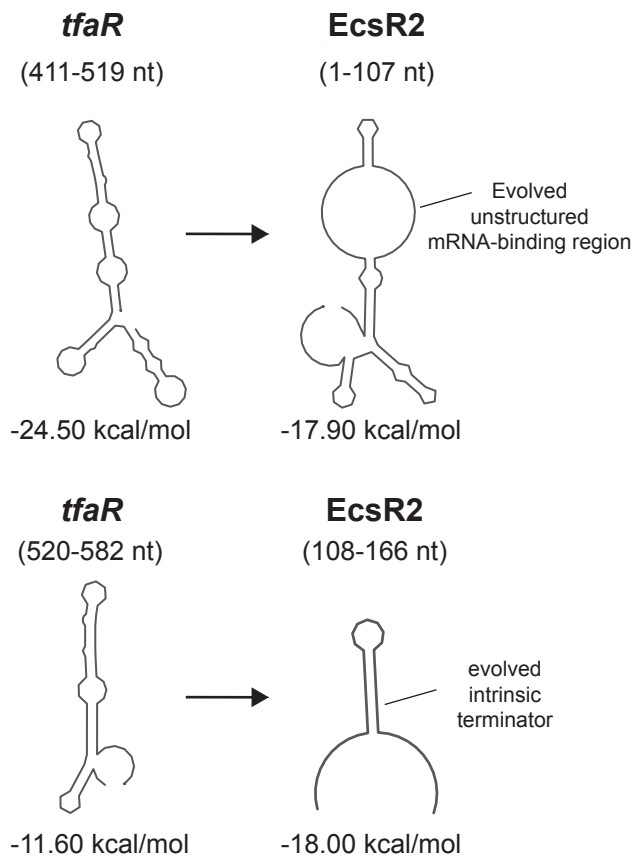


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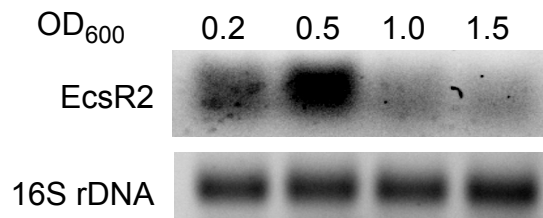


Fig. S4. EcsR2 expression. EcsR2 was measured using semi-quantitative PCR (20 cycles). 16S rRNA gene was used as a control.