

Thompson and Gottesman

SUPPLEMENTARY MATERIAL

Table S1: Strain List

Table S2: Plasmid List

Table S3: Oligonucleotide List

Figure S1: MiaA effect on *rpoS750-lacZ* translational fusion activity**TABLE S1: - Strain List**

| Strain | Genotype | Source |
|---------------|--|-----------------------------|
| AB003 | MG1655 P_{BAD^-} <i>rpoS990::lacZ kan rssB::tet</i> | (1) |
| CRB316 | MG1655 P_{BAD^-} <i>rpoS990::lacZ kan</i> | (2) |
| MG1655 | <i>Escherichia coli</i> K12 | Lab strain |
| DJ480 | MG1655 $\Delta lacX74$ | (3) |
| EM1050 | DJ480 <i>rpoS750-lacZ</i> translational fusion | (4) |
| KMT2 | MG1655 $\Delta truA::kan$ | Nancy Gutgsell/J. Ofengand. |
| KMT3 | MG1655 $\Delta truB::kan$ | " |
| KMT5 | MG1655 $\Delta rsuA::kan$ | " |
| KMT6 | MG1655 $\Delta rluA::kan$ | " |
| KMT8 | MG1655 $\Delta rluC::kan$ | " |
| KMT25 | DJ480 <i>rpoS750-lacZ</i> $\Delta truA::kan$ | EM1050 x P1(KMT2) |
| KMT26 | DJ480 <i>rpoS750-lacZ</i> $\Delta truB::kan$ | EM1050 x P1(KMT3) |
| KMT27 | DJ480 <i>rpoS750-lacZ</i> $\Delta rluA::kan$ | EM1050 x P1(KMT6) |
| KMT28 | DJ480 <i>rpoS750-lacZ</i> $\Delta rluC::kan$ | EM1050 x P1(KMT8) |
| KMT30 | DJ480 <i>rpoS750-lacZ</i> $\Delta rsuA::kan$ | EM1050 x P1(KMT5) |
| KMT31 | DJ480 <i>rpoS750-lacZ</i> <i>miaA::kan</i> | EM1050 x P1(TX2559) |
| KMT54 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>miaA::kan</i> pBAD24 | EM1050 + pBAD24 |
| KMT55 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>miaA::kan</i> /pKMT1 | EM1050 + pKMT1 |
| KMT56 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>miaA::kan</i> /pKMT2 | EM1050 + pKMT2 |

| | | |
|--------|--|------------------------------|
| KMT61 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>Δhfq::cat</i> | EM1050 x P1(YN585) |
| KMT62 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>Δhfq::cat</i> pBAD24 | KMT61 + pBAD24 |
| KMT63 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>Δhfq::cat</i> pKMT1 | KMT61 + pKMT1 |
| KMT64 | DJ480 <i>rpoS750-lacZ</i> translational fusion <i>Δhfq::cat</i> pKMT2 | KMT61 + pKMT2 |
| KMT69 | <i>rpoS750-lacZ</i> translational fusion pBAD24 | EM1050 + pBAD24 |
| KMT70 | <i>rpoS750-lacZ</i> translational fusion pKMT1 | EM1050 + pKMT1 |
| KMT71 | <i>rpoS750-lacZ</i> translational fusion pKMT2 | EM1050 + pKMT2 |
| KMT75 | MG1655 <i>rpoS::Tn10</i> <i>ΔclpP::cat</i> pBAD24- <i>rpoS</i> | Caroline Ranquet-Brazzolotto |
| KMT80 | MG1655 <i>rpoS::Tn10</i> pBAD24- <i>rpoS</i> | Caroline Ranquet-Brazzolotto |
| KMT83 | MG1655 <i>rpoS::Tn10</i> <i>miaA::kan</i> pBAD24- <i>rpoS</i> MG1655 <i>miaA::kan</i> | KMT80 x P1(TX2559) |
| KMT99 | <i>rpoS::Tn10 ΔclpP::cat</i> pBAD24- <i>rpoS</i> | KMT75 x P1(TX2559) |
| KMT581 | MG1655 P _{BAD} ⁻ <i>rpoS990::lacZ kan hfq::cat</i> | CRB316 x P1(YN585) |
| KMT582 | MG1655 P _{BAD} ⁻ <i>rpoS990::lacZ kan</i> <i>miaA::cat</i> (Nrul) | CRB316 x P1(TX4610) |
| KMT584 | MG1655 P _{BAD} ⁻ <i>rpoS990::lacZ kan rssB::tet</i> <i>miaA::cat</i> | AB003 x P1(TX4610) |
| TX2559 | MG1655 <i>miaA::kan</i> | Malcolm Winkler |
| TX4610 | W3110 <i>miaA::cat</i> (Nrul) | Malcolm Winkler |
| YN585 | MG1655 <i>Δhfq::cat</i> | YanNing Zhou |

Table S2: Plasmid List

| <u>Plasmid</u> | <u>Characteristics and markers</u> | <u>Reference</u> |
|-----------------------|--|-------------------------|
| pBAD24 | <i>araBAD</i> promoter, ColE1 ori, <i>bla</i> , AraC | (5) |
| pKMT1 | <i>miaA</i> gene cloned into EcoRI / PstI digest of pBAD24 | This work |
| pKMT2 | <i>hfq</i> gene cloned into EcoRI / PstI digest pBAD24 | This work |

Table S3: Oligonucleotide List

| <u>Oligonucleotide</u> | <u>Nucleotide Sequence (5'-3')</u> |
|-------------------------------|---|
| KT01 | ctagttgaattcaccatgagtgatatcagtaaggcgagc |
| KT02 | ctagttctgcagtcagcctgcatagaccaacaac |
| KT03 | ctagttgaattcaccatggctaaggggcaatcttta |
| KT04 | ctagttctgcagttattcggttcttcgctgctc |

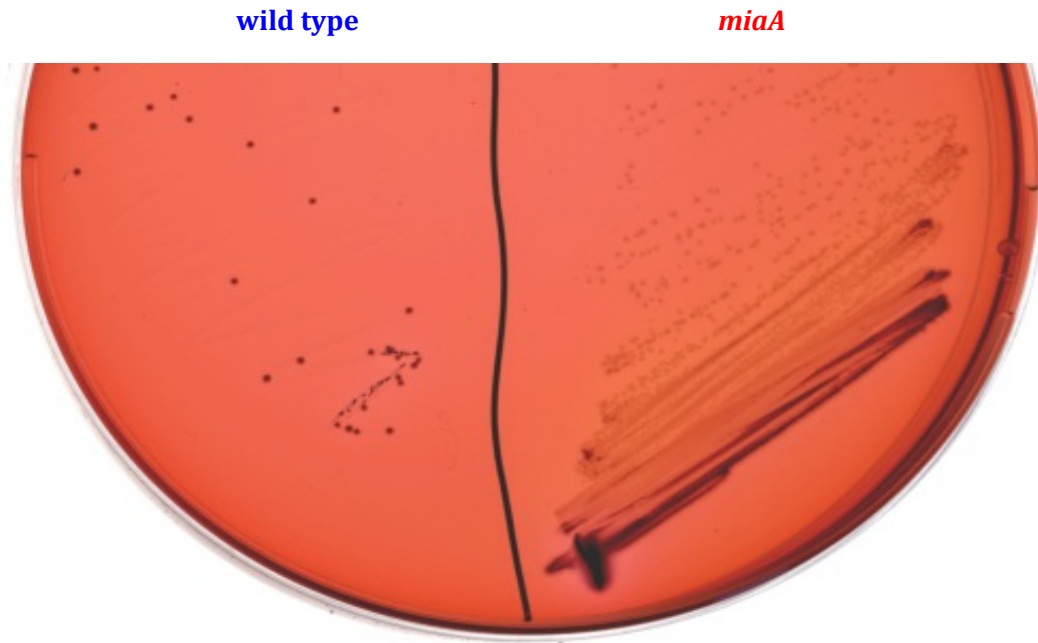


Figure S1. MiaA effect on *rpoS750-lacZ* translation fusion activity. Single colonies of wild type (EM1050) and *miaA* (KMT31) strains of *rpoS750-lacZ* translational fusion strains were picked and re-streaked on MacConkey-Lactose Agar Plates and incubated at 37°C overnight.

REFERENCES

1. **Bougdour A, Wickner S, Gottesman S.** 2006. Modulating RssB activity: IraP, a novel regulator of σ^S stability in *Escherichia coli*. *Genes and Development* **20**:884-897.
2. **Ranquet C, Gottesman S.** 2007. Translational regulation of the *Escherichia coli* stress factor RpoS: a role for SsrA and Lon. *J. Bacteriol* **189**:4872-4879.
3. **Cabrera JE, Jin DJ.** 2001. Growth phase and growth rate regulation of the rapA gene, encoding the RNA polymerase-associated protein RapA in *Escherichia coli*. *J. Bacteriol* **183**:6126-6134.
4. **Majdalani N, Hernandez D, Gottesman S.** 2002. Regulation and mode of action of the second small RNA activator of RpoS translation, RprA. *Molecular microbiology* **46**:813-826.
5. **Guzman LM, Belin D, Carson MJ, Beckwith J.** 1995. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J. Bacteriol* **177**:4121-4130.