Chen & Gottesman

Supplemental Materials

Figure S1.

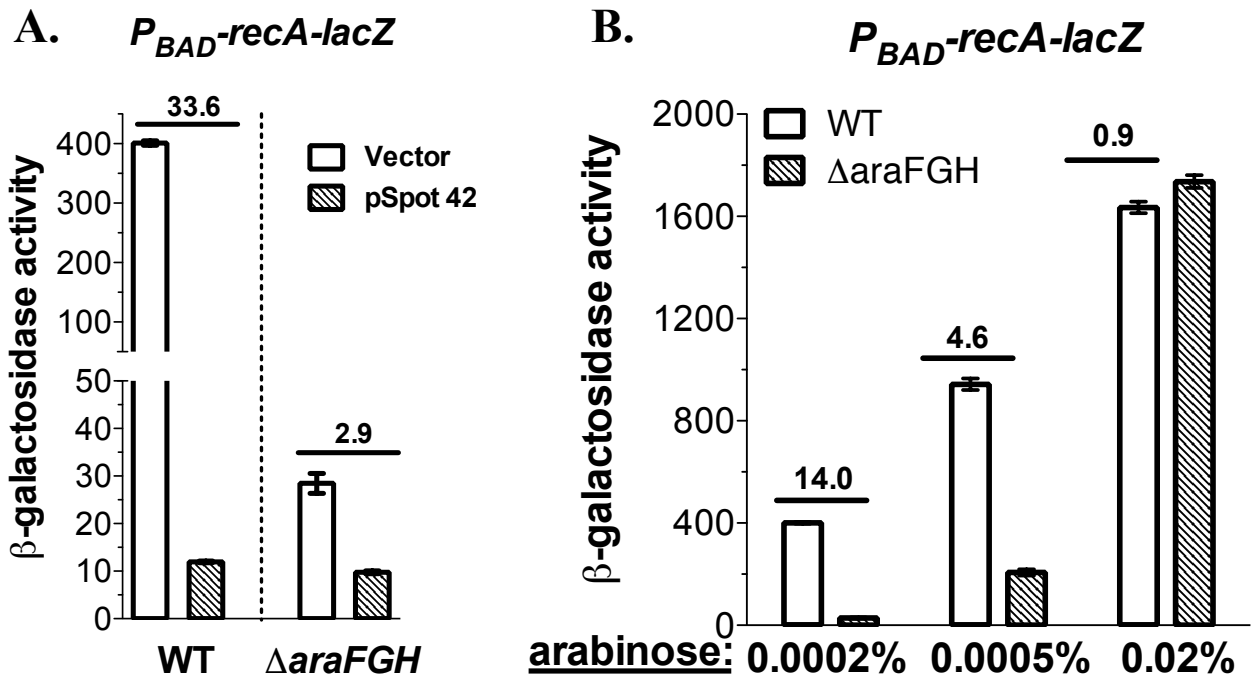

Figure S1. Effect of deleting *araFGH* on P_{BAD} induction and Spot 42 repression. β-galactosidase activity assays to measure *P_{BAD}-recA-lacZ* expression in WT (JC1005) and Δ*araFGH* (JC1180) with either Spot 42 overproduction from the plasmid in the presence of 0.0002% L-arabinose and 100 μM IPTG **(A)** or with varying doses of arabinose **(B)**. Bacterial cells were induced for 6 hrs at 37 °C before assaying for β-galactosidase activity. Biological triplicates were assayed and data are plotted as mean ± SEM, given in Miller Units.

Figure S2.

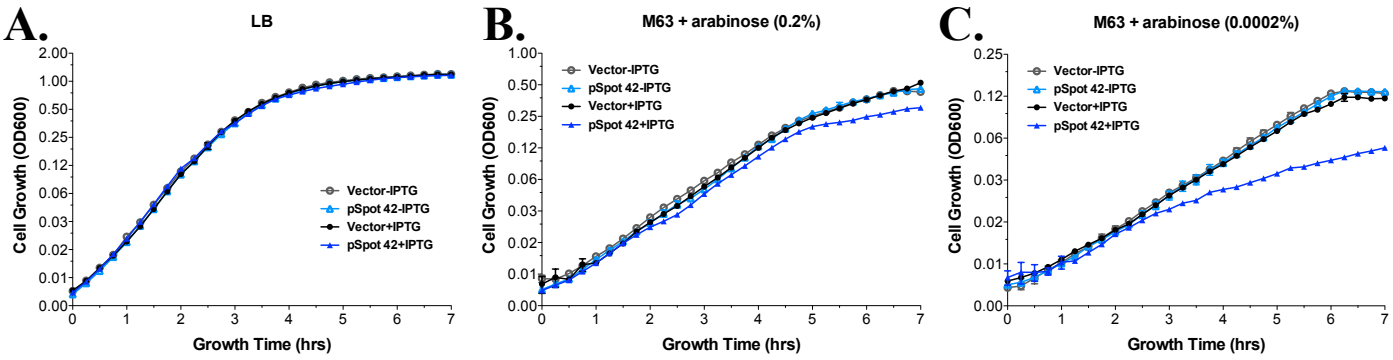

Figure S2. Overexpression of Spot 42 limits bacterial cell growth in low arabinose minimal medium. NM525 Δ*spf::cat* (JC1041) cells transformed with plasmid vector pBRplac or pSpot 42 were grown for 6 hrs in LB or in casamino acid-enriched M63 plus arabinose (0.2%) before subculturing into LB (**A**) or M63 plus arabinose (0.2% (**B**) or 0.0002% (**C**)) respectively. Bacterial cultures were started at an OD_{600} of ~0.01 with or without 100 μM IPTG induction, and growth was monitored at 37 ℃ in 24-well plates for 15 hrs using the TECAN 10M plate reader. Cell growth was determined very 15 minutes and only time points before reaching stationary phase were shown (7 hrs). Two independent experiments were conducted and data were plotted as the mean value with standard error. Note the low final OD_{600} for cells growing in 0.0002% arabinose.

Figure S3.

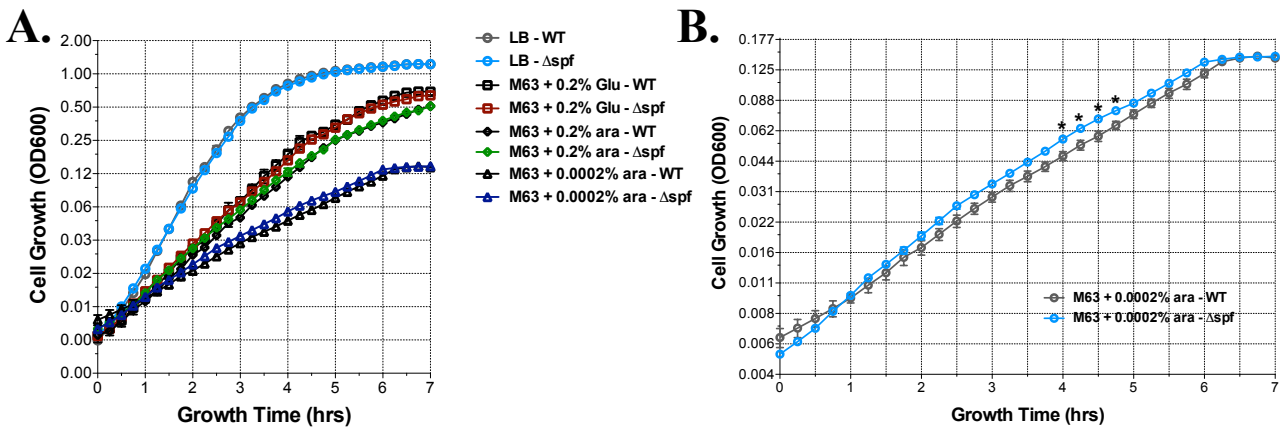

Figure S3. Endogenous Spot 42 modestly affects bacterial cell growth transition from glucose minimal medium to low arabinose minimal medium. (A). NM525 WT and Δ*spf::cat* (JC1041) cells were grown 6 hrs in casamino acid-enriched M63 plus glucose (0.2%) before subculturing into LB or M63 plus 0.2% glucose, or arabinose (0.0002% or 0.2%). Bacterial cultures were started at an OD_{600} of ~0.01, and growth monitored at 37 ℃ in 24-well plates with shaking for 15 hrs using the TECAN 10M plate reader. Cell growth was captured very 15 minutes and time points before reaching stationary phase are shown (7 hrs). **(B)**. Separate graph for bacterial cells grown in M63 + 0.0002% arabinose. Unpaired student t test was used to calculate statistical difference (* represents $p<0.05$). Two independent experiments were conducted and data were plotted as the mean value with standard error.

Supplemental tables

Table S1: Strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Strains		
MG1655	*Wild type E. coli K-12*	Lab strain collection
PM1805	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-cat-sacB-lacZ , miniλ-tetR*	(1)
NM525	*MG1655 lacI^{Q} FLP-scar*	(2)
NM543	*MG1655, lacI^{Q} FLP-scar, miniλ::tet PlacO-kan-sacB-lacZ*	(3)
NM18	*W3110 Δspf::cat*	(4)
BW27750	*BW25113 Δ(araFGH) Φ(ΔaraEp kan P$_{CP18}$–araE)*	(5)
JCS1001	*MC4100 ΔybeW::kan*	Ghigo JM
JC1005	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ*	This study[1]
JC1041	*MG1655 lacI^{Q} Δspf::cat*	NM525 + P1 (NM18)
JC1051	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::kan-CP12b-yhcN-lacZ*	J. Chen
JC1058	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::kan-CP12b-recA-lacZ*	This study[1]
JC1100	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::zeo-CP12b-araF-lacZ*	This study[1]
JC1102	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::zeo-CP12b-araF*-lacZ*	This study[1]
JC1109	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::zeo-CP12b-araF-lacZ Δspf::cat*	JC1100 + P1 (NM18)
JC1110	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::zeo-CP12b-araF*-lacZ Δspf::cat*	JC1102 + P1 (NM18)
JC1095	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ CP-araE-kan*	JC1005 + P1 (BW27750)
JC1180	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ ΔaraFGH::kan*	This study[1]
JC1186	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ araF'-kan-sacB, pSIM6*	This study[1]
JC1191	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ wild-type araF*	This study[1]
JC1192	*MG1655 mal::lacI^{Q} ΔaraBAD araC+ lacI'::PBAD-recA-lacZ araF**	This study[1]
Plasmids		
pSIM6	*Miniλ recombineering plasmid, pSC101 origin, repA^{ts}, Ampr*	(6)
pBRplac	*Parental plasmid containing Plac promoter, pBR322 origin, Ampr*	(7)
pSpot 42 - WT	*Wild type spf gene cloned into pBRplac AatII/EcoRI*	(8)
pSpot 42 - I	*mutant spf (G5A G6C T7G) cloned into pBRplac AatII/EcoRI*	(8)
pSpot 42 - II	*mutant spf (T23A C24G T25A) cloned into pBRplac AatII/EcoRI*	(8)
pSpot 42 - II'	*mutant spf (T31A C32G A33T) cloned into pBRplac AatII/EcoRI*	(9)
pSpot 42 - III	*mutant spf (G49C T50A A51T) cloned into pBRplac AatII/EcoRI*	(8)
pSpot 42 - III'	*mutant spf (G55C G56A A57C) cloned into pBRplac AatII/EcoRI*	(9)
pSpot 42 - I&III'	*mutant spf (G5A G6C T7G G55C G56A A57C) cloned into pBRplac AatII/EcoRI*	This study[1]

1. Described in Materials and Methods

Table S2: Primers, probes and synthetic gene fragments used in this study

Primers, probes and synthetic gene fragments	DNA sequence (40 nt homologous sequence for recombineering in bold)	Reference or source
Primers		
Spot 42-III'.fwd	CACGTAATCCACTTTGGCTGAATATTTTAGCCGCCCCAGTCAG	(9)
Spot 42-III'.rev	ATTCAGCCAAAGTGGATTACGTGAAGTAAAAGGTCTGAAAGATAG	(9)
JC33	**ACCTGACGCTTTTTATCGCAACTCTCTACTGTTTCTCCAT**CAACAGAACA TATTGACTAT	This study
JC28	**TAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC**CGCTTTCTGT TTGTTTTCGT	This study
JC63	**AACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACC**AAAGCCACG TTGTGTCTCAA	This study
JC71	**GTCATGCCGGGTAATACCGGATAGTCAATATGTTCTGTTG**TCAGGTATTA TATCATTTTG	This study
JC149	**TCATTCGTTTTTGCCCTACACAAAACGACACTAAAGCTGG**AAAGCCACGT TGTGTCTCAA	This study
JC150	**GACAGTGCGTTTCGCTTTTTGCTTGTAACGGTCGAAGA**GCGCTGAGGTCT GCCTCGTG	This study
JC163	**AATTTGTGCATGGTTCTCTCCAGCTTTAGTGTCGTTTTGT**ACTGTCCATAT GCACAGATG	This study
JC164	ACACAGTCACTTATCTTTTAG	This study
JC165	GGCTGCCAGGGCTTTAGTA	This study
JC167	**GCTTTGTTTTCCGATTAATTTAACGAATGTCATTCGTTTT**AAAGCCACGTT GTGTCTCAA	This study
Probes		
recA	CTGTTTGTTTTCGTCGATAGCCATTTTTACTCCTGTCATG	This study
Spot 42	GAAGTAAAAGGTCTGAAAGATAGAACATCTTACCTC	(10)
SsrA	CGCCACTAACAAACTAGCCTGATTAAGTTTTAACGCTTCA	(10)
Gene fragments		
uPBAD-zeo-CP12b-araF-lacZ	**GTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACC**TCAGTCCT GCTCCTCGGCCACGAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGA ACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGC GTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCC AGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCT GGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGT CGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGA CCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTT GGCCATGGTTTAGTTCCTCACCTTGTCGTATTATACTATGCCGATATACTATG CCGATGATTAATTGTCAACCATATACAAGTTTATTCTTGACACTAGTCGGCCA AAATGATATAATACCTGAACACAGTCACTTATCTTTTAGTTAAAAGGTAATG CTTTGTTTTCCGATTAATTTAACGAATGTCATTCGTTTTTGCCCTACACAAAA CGACACTAAAGCTGGAGAGAACCatgCACAAATTTACTAAAGCCCTGGCAGCC **GTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTA**	This study
uPBAD-zeo-CP12b-araF-lacZ*	**GTAACCCCGCTTATTAAAAGCATTCTGTAACAAAGCGGGACC**TCAGTCCT GCTCCTCGGCCACGAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGA ACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGC GTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCC AGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCT GGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGT CGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGA CCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTT GGCCATGGTTTAGTTCCTCACCTTGTCGTATTATACTATGCCGATATACTATG CCGATGATTAATTGTCAACCATATACAAGTTTATTCTTGACACTAGTCGGCCA AAATGATATAATACCTGAACACAGTCACTTATCTTTTAGTTAAAAGGTAATG CTTTGTTTTCCGATTAATTTAACGAATGTCATTCGTTTTTtgtCTACACAAAACG ACACTAAAGCTGGAGAGAACCatgCACAAATTTACTAAAGCCCTGGCAGCC**G** **TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTA**	This study

References:

1. **Lee HJ, Gottesman S.** 2016. sRNA roles in regulating transcriptional regulators: Lrp and SoxS regulation by sRNAs. Nucleic Acids Res doi:10.1093/nar/gkw358.
2. **Thomason MK, Fontaine F, De Lay N, Storz G.** 2012. A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in Escherichia coli. Mol Microbiol **84:**17-35.
3. **Lalaouna D, Morissette A, Carrier MC, Masse E.** 2015. DsrA regulatory RNA represses both hns and rbsD mRNAs through distinct mechanisms in Escherichia coli. Mol Microbiol **98:**357-369.
4. **Moon K, Gottesman S.** 2011. Competition among Hfq-binding small RNAs in Escherichia coli. Mol Microbiol **82:**1545-1562.
5. **Khlebnikov A, Datsenko KA, Skaug T, Wanner BL, Keasling JD.** 2001. Homogeneous expression of the P(BAD) promoter in Escherichia coli by constitutive expression of the low-affinity high-capacity AraE transporter. Microbiology **147:**3241-3247.
6. **Datta S, Costantino N, Court DL.** 2006. A set of recombineering plasmids for gram-negative bacteria. Gene **379:**109-115.
7. **Guillier M, Gottesman S.** 2006. Remodelling of the Escherichia coli outer membrane by two small regulatory RNAs. Mol Microbiol **59:**231-247.
8. **Beisel CL, Storz G.** 2011. The base-pairing RNA spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in Escherichia coli. Mol Cell **41:**286-297.
9. **Beisel CL, Updegrove TB, Janson BJ, Storz G.** 2012. Multiple factors dictate target selection by Hfq-binding small RNAs. EMBO J **31:**1961-1974.
10. **Mandin P, Gottesman S.** 2010. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. EMBO J **29:**3094-3107.