Supplementary Information

The AfaR small RNA controls expression of the AfaD-VIII invasin in pathogenic *Escherichia coli* strains

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Running title: Control of AfaD invasin expression.

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Supplementary Tables & Figures

Supplementary Figure S1: Relative levels of expression of the *afaABCDEFR* genes from eight *afa-8*-carrying *E. coli* strains analyzed by qRT-PCR. The AL10, AL213, AL511, 239KH89, 183, BW25113 + pILL1320, BW25113 + pILL1322 and BW25113 + pILL1323 strains were grown to an OD_{600} of 0.6 in LB medium. We set *afaA* expression levels to 1 and the expression levels of other genes are expressed relative to *afaA*.

Supplementary Figure S2: Results from circular RACE experiments on the *afaC* mRNA. (A) Position of ribonuclease cleavages (gray arrow) in the *afaB*/*afaC* intergenic region. (B) Position of ribonuclease cleavages (gray arrow) in the *afaC*/*afaD* intergenic region. The "Met" tag and asterisks indicate the start and stop codons of the CDS, respectively.

Supplementary Figure S3: RNase III/LS/G/P dependence in the regulation of *afaA, afaC* and *afaD* mRNAs and AfaR sRNA. (A) Relative expression of the *afaA, afaC, afaD* mRNAs and AfaR was determined by qRT-PCR in RNase-depleted strains with respect to the corresponding wild-type strains, both carrying the pILL1320 plasmid. A minimal two-fold change in the relative level indicated a significant implication of the tested RNase (symbolized by dashed lines). (B) Model of the posttranscriptional regulation of the *afa-8* gene cluster by RNases and AfaR sRNA. The open arrows indicate the position of cleavage sites.

Supplementary Table 1:

Amplification	Name	Primer sequence
Allelic exchange		
afaR ::KmFRT	afaR.FRT.5	5‘-CATCAAAAAGTGGACGTCTGTTATGGAACATATGTAGCTAGTGTAGGCTG GAGCTGCTTC-3’
	afaR.FRT.3	5’-GTGATATTTTAATGTACAGTCAACTACAACAGTAGCTGCCCATATGAATA TCCTCCTTA-3’
afaRE ::KmFRT	afaRE.FRT.3	5’-GCCCCCTTCTTTCATCAGGTTAATTTTCCATCAGTCGGTCCATATGAATAT CCTCCTTA-3’
afaD ::KmFRT	afaD.FRT.5	5’-TCTGGTGCGCACTGTGTGTATGTGACCAAGGAGTACCCTGGTGTAGGCTG GAGCTGCTTC-3’
	afaD.FRT.3	5’-ACTGAATAGTTACTTTATTAGCATAAAGAAATTATGAATACATATGAATA TCCTCCTTA-3’
afaFA ::KmFRT	afaPrA.FRT.5	5’-ACGGAGTTTTCAGCTCCGTTAGGAACGTCAGTATATTGTCTTTCATGTGTA GGCTGGAGCTGCTTC-3’
	afaPrA.FRT.3	5’-TAACCCCGTTTACTCTGACAGGGAAACAAACTCTCAGCATCATATGAATA TCCTCCTTA-3’
Allelic exchange verification		
afaR ::KmFRT	afaR.verif5	5’-TTGGTGATGCAGTGTCCACC-3’
	afaR.verif3	5’-TCTATAATACTGCTTCACCAC-3’
afaD ::KmFRT	afaD.verif5	5’-GAGCGGACTGGATGAAACCG-3’
	afaD.verif3	5’-GCTATTTTAGAGGGCAGCCA-3’
afaFA ::KmFRT	afaPrA.verif5	5’-CATCAGGTAATATCTGGCCT-3’
	afaPrA.verif3	5’-CACTTTCTCACTGTGCATCC-3’
Cloning		
afa8	clon.afa8.5	5’-CCCGGGAAGCTTAATGGCATGGTTCATCGTCT-3’
	clon.afa8.3	5’-CCCGGGAAGCTTGCCAGGCTGATTTAAACACAA-3’
afaR	Cl.afaR.EcoRI	5’-TTTTTTGAATTCTAGCTACATATGTTCCATAACAGAC-3’
	Cl.afaR.XbaI	5’-TTTTTTTCTAGACAGTCTGGCGCAGGCAGTTG-3’
afaD::gfp	afaC.NsiI	5’-GTTTTTATGCATAATTATCGACTTCCAGAGGAG-3’
	afaD.NheI	5’-GTTTTTGCTAGCCGCCAGACTGGATATAACCAC-3’
lacZ ::afaD	afaD NsiI	5’-TGCATGGAAATCAGCTGCTTGGGCCTGATG-3’
	afaD.XbaI	5’-GTTTTTTCTAGACCATAACAGACGTCCACTTT-3’
*afaR**	mutafaR5	5’-CTTACATTgtATATGCATTTTAAC-3’
	mutafaR3	5’-GTTAAAATGCATATacAATGTAAG-3’
afaD::gfp*	mutafaCD5	5’-TGGTCAgtTACACACAGTGC-3’
	mutafaCD3	5’-GCACTGTGTGTAacTGACCA-3’
afaA::lacZ	pQFafaA.XbaI	5’-CCCGGGTCTAGACAGCGCCGGAATGAACACC-3’
	pQFafaA.XhoI	5’-CCCGGGCTCGAGTTTTTGATTAATGTCCAGAAAATAAGA-3’
afaB::lacZ	pQFafaB.XbaI	5’-CCCGGGTCTAGATATTTTATCCTGAAGGCAAA-3’
	pQFafaB.XhoI	5’-CCCGGGCTCGAGTCAGGGAGCATCTTGTTATGG-3’
afaC::lacZ	pQFafaC.XbaI	5’-CCCGGGTCTAGATATTCACACCTGAGCCACGG-3’
	pQFafaC.XhoI	5’-CCCGGGCTCGAGTGGTGCATCAGGAAAAGTCA-3’
afaD::lacZ	pQFafaD.XbaI	5’-CCCGGGTCTAGACAGGGTACTCCTTGGTCACA-3’
	pQFafaD.XhoI	5’-CCCGGGCTCGAGACTCGAGTAGGGGGAAGAGC-3’
afaE::lacZ	pQFafaE.XbaI	5’-CCCGGGTCTAGAACCTTATCCTTATACTTGGT-3’
	pQFafaE.XhoI	5’-CCCGGGCTCGAGCAGTGTCCACCTTTTCATCA-3’
afaR::lacZ	pQFafaR0.XbaI	5’-TTTAAACTCGAGTTTAAATGCTTCCACCAGTG-3’
	pQFafaR0.XhoI	5’-CCCGGGTCTAGAAATGTAAGTGTAAACTGAGTGCCG-3’
Cloning verification		
afaR	verif.pZE2R.5	5’-AATAGGCGTATCACGAGGCC-3’
	verif.pZE2R.3	5’-GTCGACCTGCAGCTAGGTCT-3’
afaD fusion	pZE-CAT	5’-TGGGATATATCAACGGTGGT-3’
	JV0155	5’-CCGTATGTAGCATCACCTTC-3’
afa ::lacZ fusion	pQF50.5	5’-CGACTCCTGCATTAGGAAGC-3’
	pQF50.3	5’-GTTTTCCCAGTCACGACGTT-3’
Quantitative RT-PCR		
5S	5S.Fw	5’-GGTGGTCCCACCTGACC-3’
	5S.RT	5’-ATGCCTGGCAGTTCCCTACT-3’
afaA	afaA.Fw	5’-CCGGGATGCACAGTGAGAAA-3’
	afaA.RT	5’-AACACCGTGCTGCTCACAGG-3’
afaB	afaB.Fw	5’-GAGCCGTTTGCGAATTGTCC-3’
	afaB.RT	5’-CATCATGCTTTGGCGGAATG-3’
afaC	afaC.Fw	5’-CTGCTGAACTGGCAGGCAAA-3’
	afaC.RT	5’-ATGCCCGGCTCAAGAGTGAC-3’
afaD	afaD.Fw	5’-CAGTCTGGCGCAGGCAGTT-3’
	afaD.RT	5’-CCTTCCTGTTGCCACCTTCG-3’
afaE	afaE.Fw	5’-AGACTTCGCCAGGACCGTTG-3’
	afaE.RT	5’-ATCGGTGCGCCGTATGAACT-3’
afaF	afaF.Fw	5’-CCGTGCCGGACAACAGAAAT-3’
	afaF.RT	5’-CCGGCGAGCGTTTTATCTTTC-3’
afaR	afaR.Fw	5’-ATGTTCCATAACAGACGTCCAC-3’

	afaR.RT	5’-AATGTAAGTGTAAACTGAGTGCCGTA-3’
lacZ	lacZ.Fw	5’-CGTTTTACAACGTCGTGACTG-3’
	lacZ.RT	5’-GGCCTCTTCGCTATTACGC-3’
gfp	gfp.Fw	5’-TACAAGACGCGTGCTGAAGT-3’
	gfp.RT	5’-TGTGTCCGAGAATGTTTCA-3’
5’ RACE		
AfaR sRNA	pri.afaR.RT	5’-GCCATTAAACTTGTATTGGTGATGCAGTGTCC-3’
5’ afaC mRNA	rigBC.RT	5’-GTGCATTATTCACACCTGAGC-3’
3’ afaC mRNA	rigCD.Fw	5’-GAGGAGAAAGAAGCAACTGGT-3’
afaD mRNA	pri.afaD.RT	5’-CCTTCCTGTTGCCACCTTCG-3’

Supplementary Table S2: Additional strains.

Name	Description / Relevant characteristics	Phenotypes	Source / Reference
***E. coli* strains**			
A19	*rna-19, gdhA2, his-95, relA1, spoT1, metB1*		CGSC# 5997
AB301-105	A19 *rnc-105*	RNase III$^-$	CGSC# 5400
GW10	W3110 *zce-726::Tn10*		64
GW11	GW10 *cafA::cat*	RNase G$^-$	64
MH1	*sup0, hsdR, ΔlacX74, rpsL*		65
TY0324	*MH1 ΔrnlA::km*	RNase LS$^-$	65
ts709	*rnpB-709^{ts}*	RNase P^{ts}	66

CGSC: Coli Genetic Stock Center.

Supplementary results

The afaABCD mRNA is cleaved by multiple RNases

Characterization of the transcriptional units of the *afa-8* gene cluster led us to suggest that RNases might regulate the level of *afaABCD* mRNA posttranscriptionally. We tested this hypothesis, by analyzing the dependence of four *E. coli* RNases (RNase III/P/G and LS) on the expression of the *afa-8* gene cluster. The pILL1320 plasmid was introduced into *E. coli* Δ*rnc*-105 (RNase III$^-$), Δ*rnlA* (RNaseLS$^-$) and Δ*cafA* (RNase G$^-$) strains, and an *E. coli* strain carrying the thermosensitive allele *rnpB*-709 (RNase P^{ts}) and their corresponding wild-type (WT) counterparts. The WT, Δ*rnc*-105, Δ*rnlA* and Δ*cafA* strains were grown to an OD_{600} of 0.6 at 37°C. In the case of the thermosensitive *rnpB*-709 mutant, samples were obtained as described fors RNase E strains. The levels of *afaA*, *afaC*, *afaD* mRNAs and of the AfaR sRNA were determined by qRT-PCR in these strains and are expressed relative to the WT values. No significant differences in *afaA* transcription were observed between the tested mutants, suggesting that RNases had no effect on transcription (Supplementary Figure S3A). However, a significant increase in the level of transcription of *afaC* and *afaD* and *afaR* was observed when RNase III was inactivated. Interestingly, the *afaD* mRNA and the AfaR sRNA were less abundant in an RNase LS-depleted strain. Our findings do not rule out the

possibility of a significant effect of RNases G and P. The results presented in the text suggest that the RNA was cleaved around *afaC*, by an unknown RNase. We hypothesized that RNase III might be involved in *afaABCD* mRNA processing. A putative model of regulation by RNase E/III and AfaR sRNA is shown in Supplementary Figure S3B.

Supplementary References

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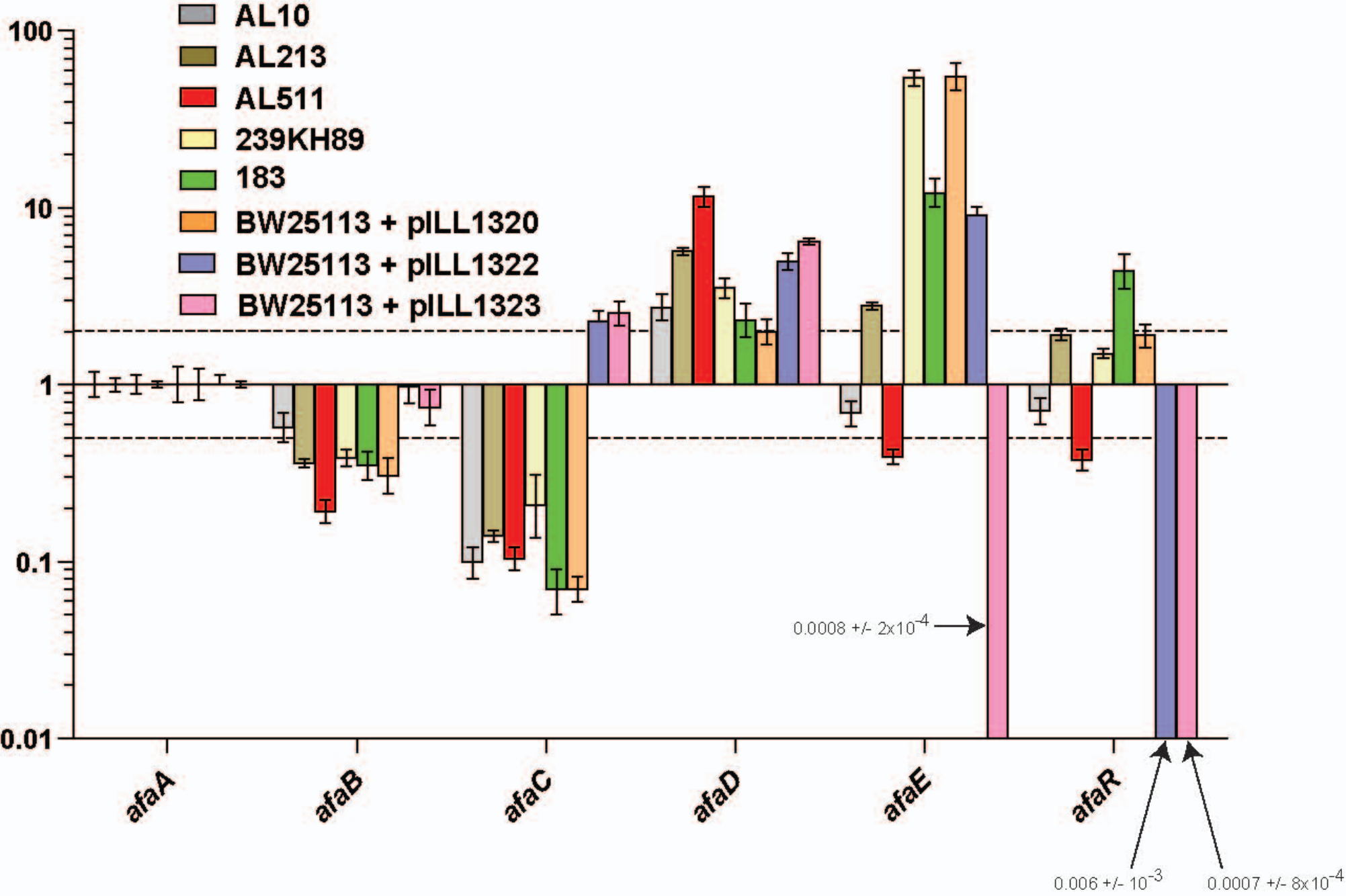
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AL213
AL511
239KH89
183
BW25113 + pILL1320
BW25113 + pILL1322
BW25113 + pILL1323
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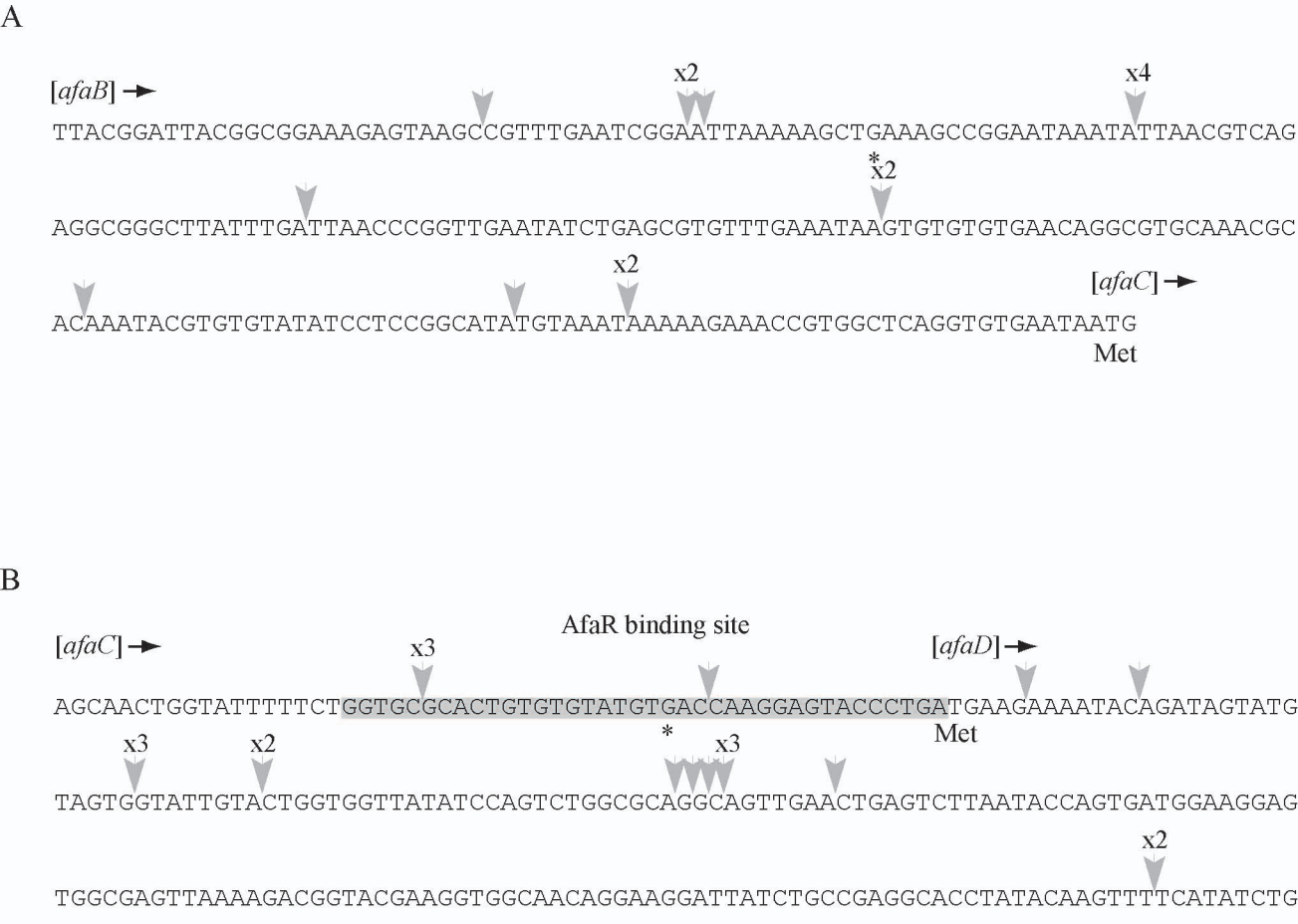
0.1
0.01
0.0008 +/- 2x10-4
0.006 +/- 10-3
0.0007 +/- 8x10-4
afaA
afaB
afaC
afaD
afaE
afaR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x4
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x2
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Met
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AfaR binding site
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x3
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Met
x3
x2
*
x3
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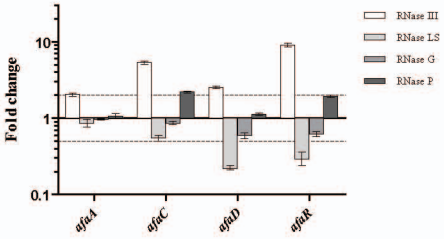
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RNase LS
RNase G
RNase P
Fold change
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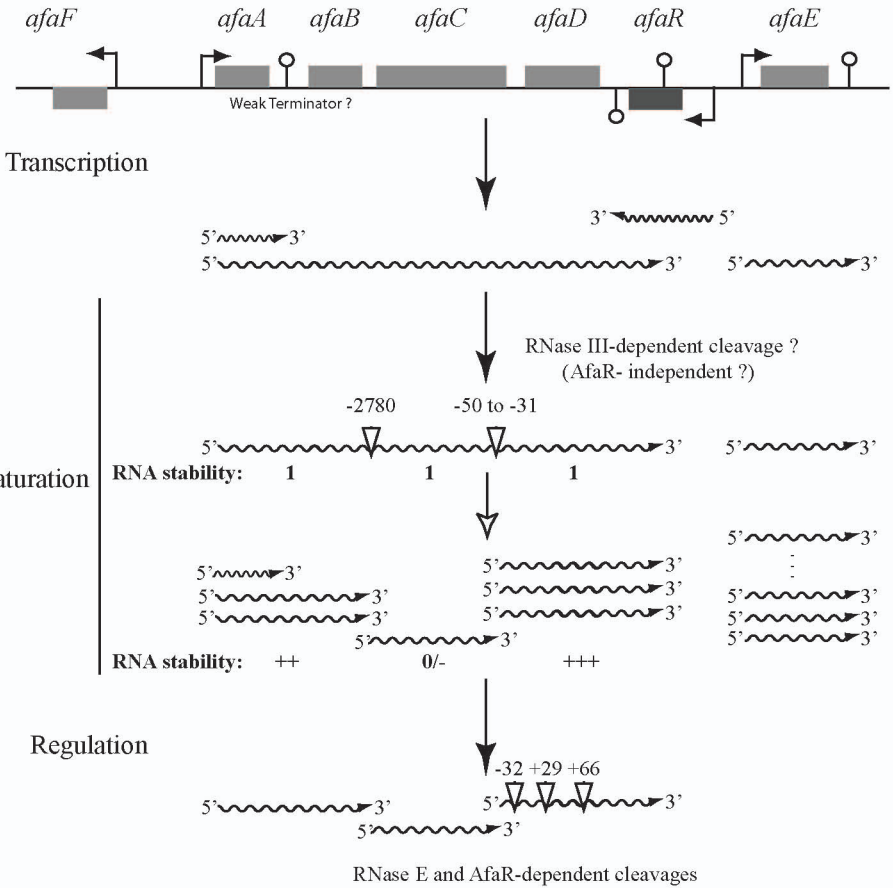
0.1
afaA
afaC
afaD
afaR

B


afaF
afaA
afaB
afaC
afaD
afaR
afaE
Weak Terminator ?
Transcription
sRNA
mRNA
RNase III-dependent cleavage ?
(AfaR- independent ?)
-2780
-50 to -31
Maturation
RNA stability:
1
1
1
RNA stability:
++
0/-
+++
Regulation
-32 +29 +66
RNase E and AfaR-dependent cleavages