

Running Title: An RNA attenuates bacterial virulence and host cell uptake.

A bacterial regulatory RNA attenuates virulence, spread and human host cell phagocytosis.

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Supplemental Tables and Figure

Table S1: Strains and plasmids used in this study

Strains	Relevant characteristics	Reference
<i>E. coli</i> strain		
<i>DH5α</i>	α F- ϕ 80 Δ <i>lacZ</i> Δ <i>M15</i> Δ (<i>lacZA-argF</i>) <i>U169 deoR</i> <i>recA1 endA1 hsdR17</i> (rk- mk-) <i>phoA supE44 λ- thi-1</i> <i>gyrA96 relA1</i>	(42) (43)
<i>S. aureus</i> strains		
<i>RN4220</i>	Restriction-defective derivative of 8325-4	
<i>Newman</i>	Methicillin sensible <i>S. aureus</i> strain isolated in 1952 from a human infection.	(44)
<i>Newman ΔsprC</i>	<i>Newman</i> strain deleted for <i>sprC</i>	This study
<i>Plasmids</i>		
pBT2	Low-copy-number shuttle vector with Amp ^R in <i>E. coli</i> and temperature-sensitive replication with Cat ^R in <i>S. aureus</i>	(19)
pCN35	High-copy-number shuttle vector with Amp ^R in <i>E. coli</i> and erm ^R in <i>S. aureus</i>	(45)
pCN38	Low-copy-number shuttle vector with Amp ^R in <i>E. coli</i> and cat ^R in <i>S. aureus</i>	(45)
pCN35 Ω <i>sprC</i>	pCN35 with <i>sprC</i> under the control of its endogenous promoter	This study
pCN38 Ω <i>sprC</i>	pCN38 with <i>sprC</i> under the control of its endogenous promoter	This study

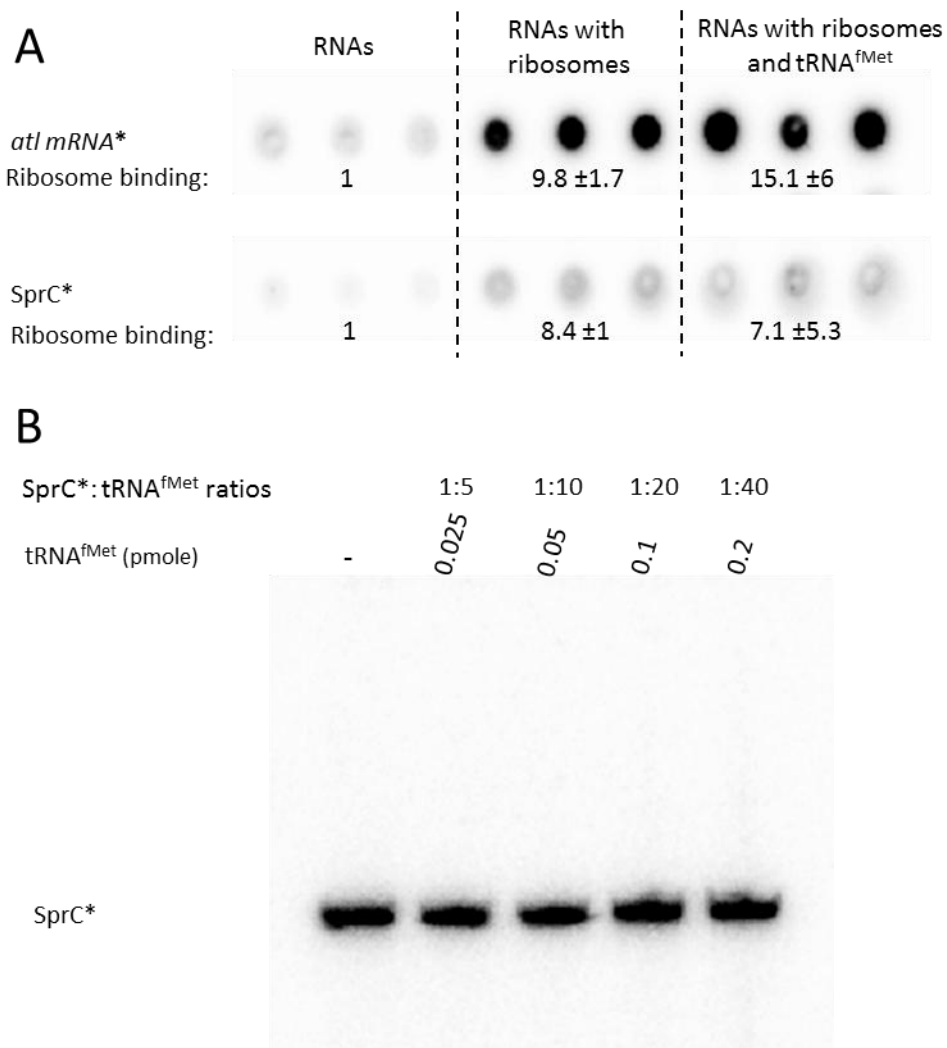
Table S2: DNA primers used in this study

DNAs	Sequences (5'-3')	Purposes
antiSprC	CGGCTACTACATTCGCATGT	Northerns SprC
SprCEcoR1	TACCGGAATTCGTATACTGTTATAACTG	pCN35- <i>sprC</i> and
SprCPst1	TAGATCTGCAGATAGGTGAGATGCATACAC	pCN38- <i>sprC</i>
Dis1	TAGATCTGCAGACTAATTGTTTTAGAATAATTAATG	<i>sprC</i> deletion
Dis2	ATTCAATTCCTAGGTGTTTCATAGTAAATATATATATG	
Dis3	ACCAGTTTTCCGCGGCGCTCGAGTATGAAAATGTTGATGAGCTA C	
Dis4	TACCGGAATTCATAAAGCGTAAATTATTAATTATC	
Dis5	CATATATATATTTACTATGAACACCTAGGAATTGAAT	
Dis6	GTAGCTCATCAACATTTTCATCTCGAGCGCCGCGGAAAATGTT	
Atl 154for	TAATACGACTCACTATAGGGAATAATTAATAAATGTTAGGA	<i>atl</i> mRNA amplification, transcription and toeprint
Atl 154rev	TATTAGTAGTTTGATCTTGTG	
SprCfor	TAATACGACTCACTATAGGGAAGTCAACGACCATGCGTGGACAG	SprC amplification and transcription
SprCrev	CCATGATTTTCGAAGTCTTCATAAACTG	
SprC _{Δ78-89} for	GTTTACTATGAGACTAGGCAATCATAGTATAAATT	SprC _{Δ78-89} amplification and transcription
SprC _{Δ78-89} rev	AATTTATACTATGATTGCCTAGTCTCATAGTAAAC	
SprC _{Δ55-67} for	AAGTACATGCGAATGTAGTAAGACTAGGCATTATAATTGA	SprC _{Δ55-67} amplification and transcription
SprC _{Δ55-67} rev	TCAATTATAATGCCTAGTCTTACTACATTCGCATGTACTT	

Table S3: Primer sequences used for qPCR

Genes Designations	Forward (5'-3')	Reverse (5'-3')
<i>tmRNA</i>	CACTCTGCATCGCCTAACAG	TCAAACGGCAGTGTTTAGCA
<i>sprC</i>	AAGCTTCTACTCTCATGGCAATTT	TGCGTGGACAGTAAAACGAA
<i>hu</i>	CCTCAAAGTTACCGAAACCAA	AGCTGGTTCAGCAGTAGATGC

Figure S1: SprC does not interact with either the *S. aureus* ribosomes or Met-tRNA. **A.** Filter binding assays showing that SprC does not bind specifically to the ribosomes set into a translation initiation mode (in the presence of tRNA^{fMet}), whereas the *atl* mRNA does. The quantifications of the dot blots are indicated below the experiments **B.** Native gel retardation assays of purified labeled SprC with increasing amounts of purified, unlabeled tRNA^{fMet}.



Supplementary references:

42. Waters,L.S. and Storz,G. (2009) Regulatory RNAs in bacteria. Cell, 136, 615–628.
43. Windbichler,N. and Schroeder,R. (2006) Isolation of specific RNA-binding proteins using the streptomycin-binding RNA aptamer. Nat. Protoc., 1, 637–640.

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44. Cheung,A.L., Eberhardt,K.J. and Fischetti,V.A. (1994) A method to isolate RNA from gram-positive bacteria and mycobacteria. *Anal. Biochem.*, 222, 511–514.
45. Hartz,D., McPheeters,D.S., Traut,R. and Gold,L. (1988) Extension inhibition analysis of translation initiation complexes. *Methods Enzymol.*, 164, 419–425.