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Valérie Bordeau, Brice Felden. Curli synthesis and biofilm formation in enteric bacteria are controlled by a dynamic small RNA module made up of a pseudoknot assisted by an RNA chaperone.. Nucleic Acids Research, Oxford University Press, 2014, 42 (7), pp.4682-96. 10.1093/nar/gku098 . inserm-00941995

HAL Id: inserm-00941995

<https://www.hal.inserm.fr/inserm-00941995>

Submitted on 4 Feb 2014

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Curli synthesis and biofilm formation in enteric bacteria is controlled by a dynamic small RNA module made up of a pseudoknot assisted by an RNA chaperone.

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Supplemental Material

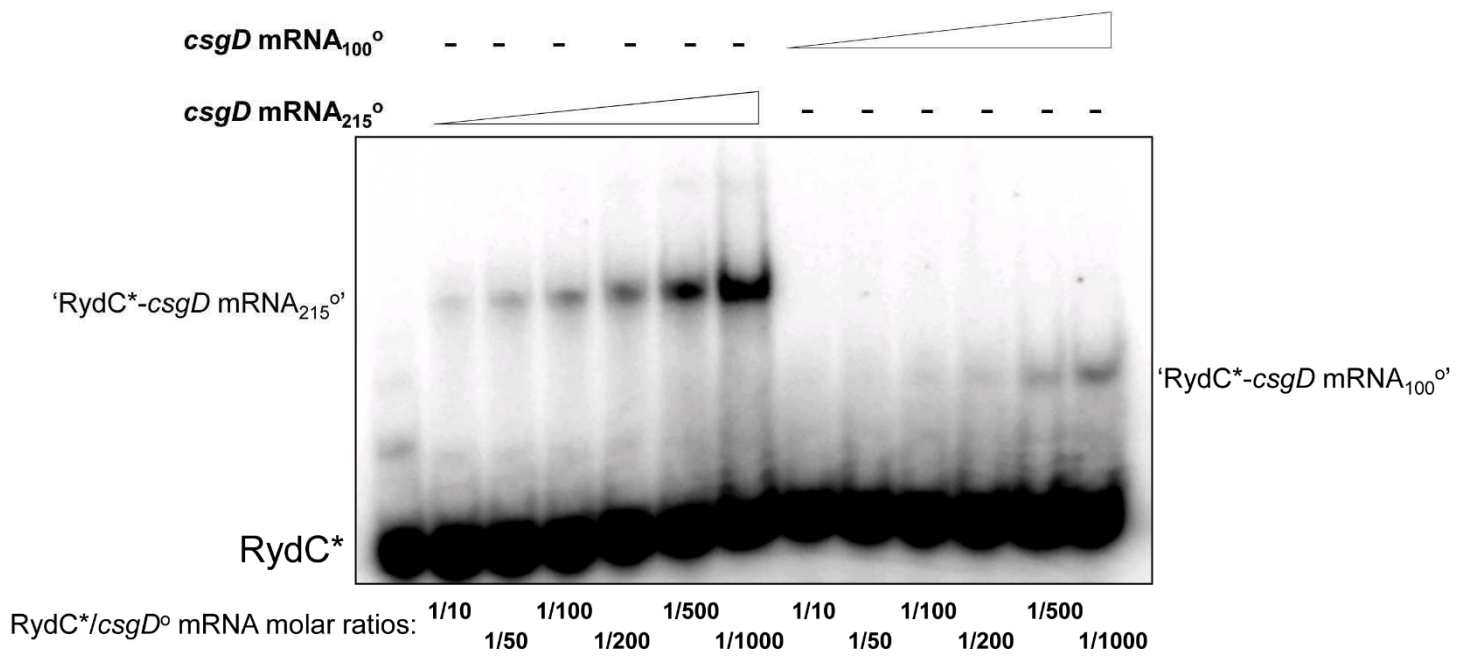


Figure S1. Complex formation between RydC and two $csgD$ mRNA fragments of different lengths. $csgD$ mRNA₁₀₀ and $csgD$ mRNA₂₁₅ correspond to 100 and 215 nts from the $csgD$ mRNA 5'-end, respectively. Native gel retardation assays of purified labelled RydC with increasing amounts of unlabelled $csgD$ mRNA₂₁₅ and $csgD$ mRNA₁₀₀ (10 to 1000-fold more than RydC) in the absence of the Hfq protein. This indicates that in the absence of the Hfq protein, the affinity between the two RNAs is weak.

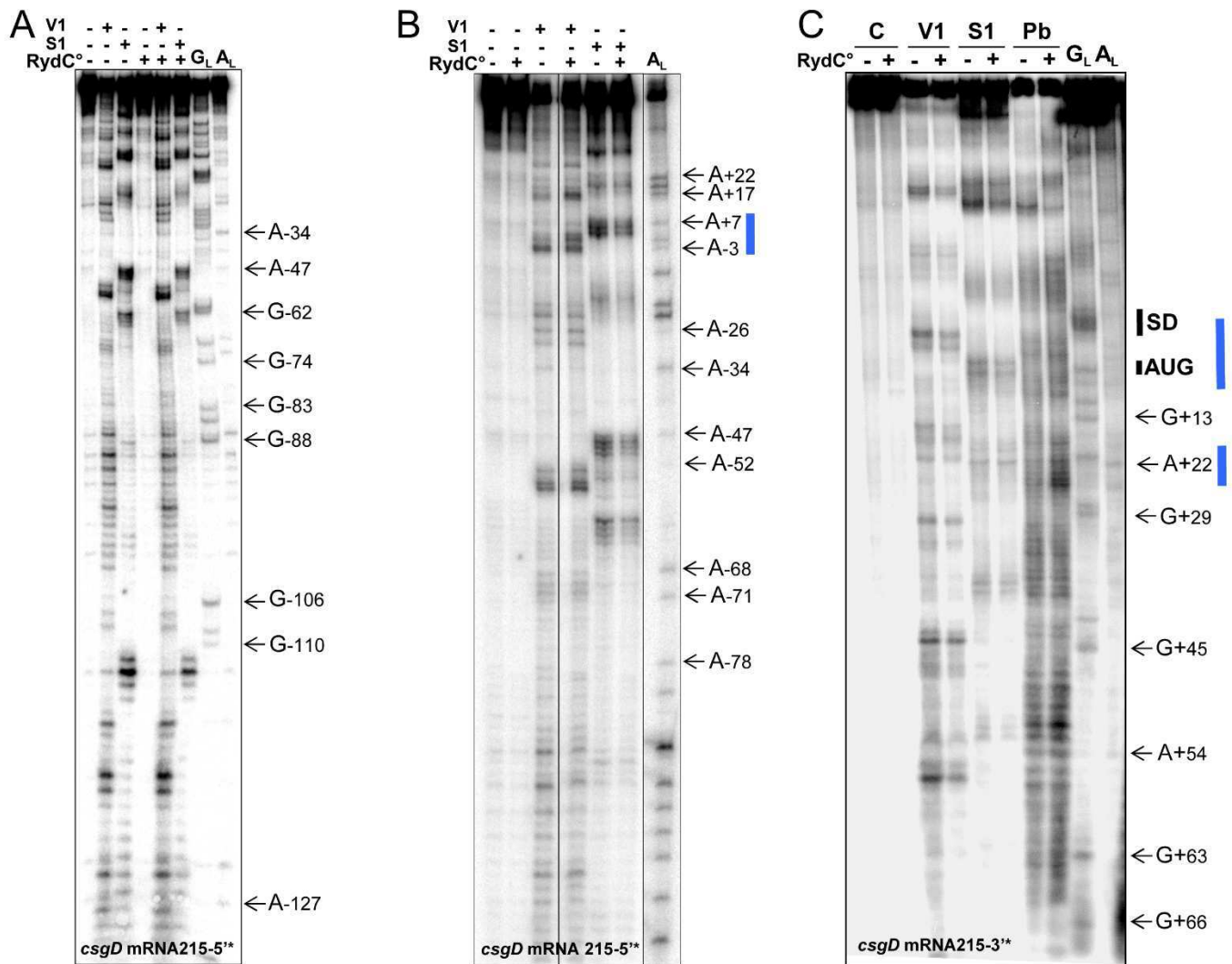


Figure S2. Structural analysis of the conformational changes in *csgD* mRNA₂₁₅ induced by complex formation with RydC. Autoradiograms of the cleavage products of 5'- (A, B) or 3'-labelled (C) *csgD* mRNA₂₁₅ by RNases V₁ ($5 \cdot 10^{-5}$ unit) and nuclease S₁ (2 units) in the presence or absence of unlabelled RydC at a 1:100 molar ratio. Lanes G_L, RNase T₁ hydrolysis ladder; lanes A_L, RNase U₂ hydrolysis ladder. The RNA sequences are indexed on the right sides of each panel. The conformational changes of the *csgD* mRNA upon complex formation with RydC are indicated with vertical blue bars. The SD sequence and AUG initiation codon of the mRNA are also shown.

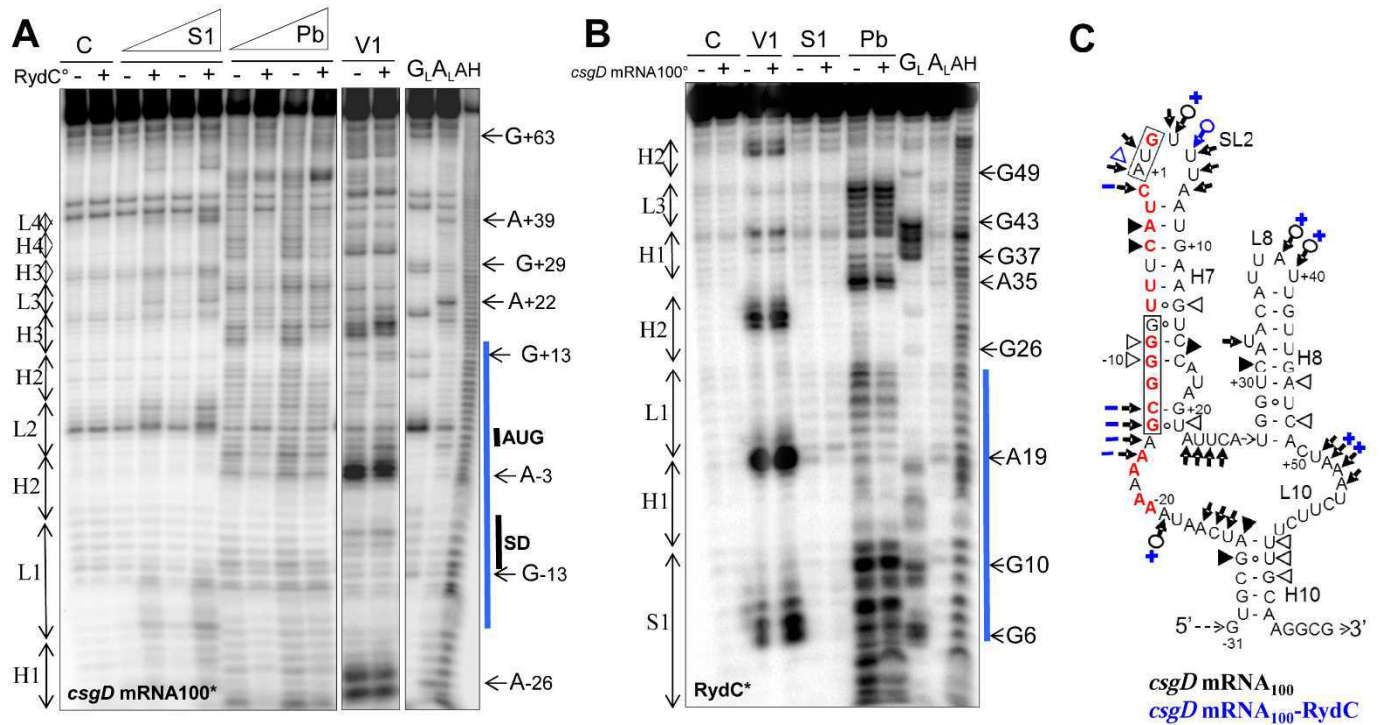


Figure S3. Structural analysis of the conformational changes of RydC and *csgD* mRNA₁₀₀ induced by complex formation with *csgD* mRNA₁₀₀ and RydC, respectively. **A, B.** Autoradiograms of cleavage products of 5'-labelled *csgD* mRNA₁₀₀ (100 nts-long) (A) or 5'-labelled RydC (B) by RNases V₁ (5.10⁻⁵ unit), nuclease S₁ (0.5 and 1 unit) and lead acetate (0.5 and 1 mM) in the presence or absence of either unlabelled RydC (A) or unlabelled *csgD* mRNA₁₀₀ (B) at 1:100 molar ratios. The *csgD* mRNA₁₀₀ or RydC structural domains are indicated on the left sides of each panel. Upon complex formation, the conformational changes of *csgD* mRNA₁₀₀ or RydC are highlighted by vertical blue bars. **C.** Secondary structure of the *csgD* mRNA₁₀₀ inferred from the probing results, which support the proposed model. Triangles are V₁ cuts; arrows capped by a circle are S₁ cuts; uncapped arrows are lead cuts. The cut and cleavage intensities are proportional to the darkness of the symbols. The structural domains are indicated and the AUG and SD sequences are outlined. The red nucleotides are those proposed to interact with RydC. Structural changes detected in the *csgD* mRNA₁₀₀ upon RydC complex formation are in blue.

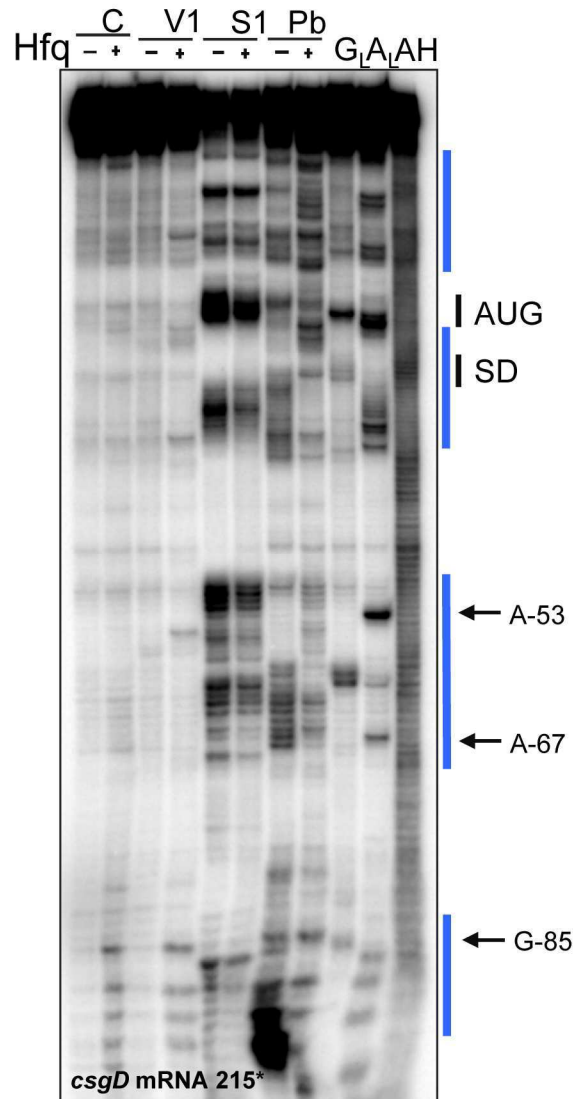


Figure S4. Structural analysis of the conformational changes of *csgD* mRNA₂₁₅ induced by complex formation with Hfq. Autoradiograms of the cleavage products of 5' *csgD* mRNA₂₁₅ by RNases V₁ ($15 \cdot 10^{-5}$ unit), nuclease S₁ (0.5 units), and lead acetate (1 mM) in the presence or absence of Hfq at a 1:20 molar ratio. Lanes G_L, RNase T₁ hydrolysis ladder; lanes A_L, RNase U₂ hydrolysis ladder. The RNA sequences are indexed on the right sides of the panels. Upon complex formation with Hfq, the conformational changes in *csgD* mRNA are emphasized by the vertical blue bars. The SD sequence and AUG initiation codon of the mRNA are indicated

Table S1. Strains used and constructed in this study.

Strain	Description	Source,Reference
<i>E. coli</i> MG1655Z1	Z1(lacR tetR SpR)	(4)
<i>E. coli</i> MG1655Z1 Δ rydC	Z1(lacR tetR SpR) Δ RNA1114::Cm	(4)
<i>E. coli</i> MG1655Z1 pUC18	MG1655Z1 + pUC18	(4)
<i>E. coli</i> MG1655Z1 pUC18-rydC	MG1655Z1 + pUC18-RNA1114	(4)
<i>S. enterica</i> subsp. <i>bongori</i> +pUC18	<i>Salmonella enterica</i> subsp. <i>bongori</i> + pUC18	This study
<i>S. enterica</i> subsp. <i>bongori</i> +pUC18-rydC	<i>Salmonella enterica</i> subsp. <i>bongori</i> + pUC18-RNA1114	This study
<i>S. sonnei</i> +pUC18	<i>Shigella sonnei</i> + pUC18	This study
<i>S. sonnei</i> +pUC18-rydC	<i>Shigella sonnei</i> + pUC18-RNA1114	This study

Table S2. DNA oligodeoxyribonucleotides used in this study.

Names	Sequences (5' → 3')	Purposes
csgD215rev	CGCCTGCAAAGAAGATTTAGT	<i>csgD</i> mRNA ₂₁₅ transcription toeprint <i>csgD</i> mRNA ₁₀₀ transcription, <i>csgD</i> mRNA ₁₁₅ transcription
csgD215for	<u>TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTT</u> ACCC	<i>csgD</i> mRNA ₂₁₅ transcription
csgD100for	<u>TAATACGACTCACTATAGGGTGCATCAATAAAAAAAGCGGGG</u> TTTCAT	<i>csgD</i> mRNA ₁₀₀ transcription
csgD503	TTGCAACCCTTAATTGACAACACGTTCTTGAT	<i>csgD</i> mRNA ₅₀₃ transcription
csgD Δ 5'UTRfor	<u>TAATACGACTCACTATAGGTTTAATGAAGTCCATAGTA</u>	<i>csgD</i> mRNA Δ 5'UTR
csgD115rev	ACCTGACAGCTGCCTCTAAAA	<i>csgD</i> mRNA ₁₁₅ transcription
csgDnorth	CAATGTCGCGGTACGGGTAATCTTCAGGCGTATTTAGCAA	<i>csgD</i> mRNA northern
RydCfor	<u>CGGGATCCTAATACGACTCACTATAGGGCTTCCGATGTAGACCC</u> GTT	RydC transcription
RydCrev	AAGAAAACGCCTGTACTAAAAC	RydC transcription
RydCnorth	ACCGACCCGTGGTACAGGCG	RydC northern
RydC Δ 5'for	<u>TAATACGACTCACTATAGGCCTGTACCACGGGTCGGTTTTAGTA</u> CAGGCGTTTTCTT	RydC transcription
RydC Δ 5'rev	AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCCTAT AGTGAGTCGTATTA	RydC Δ 5' transcription
tmRNAnorth	GTTTTAACGCTTCAACCCCA	tmRNA northern
5Snorth	CTTCTGAGTTCGGCATGGGC	5S rRNA northern
csgBAnorth	AACTGCAGCACCGTTGCCACCACCGAACTGTTTAACCGTCATTT AAT	<i>csgBA</i> mRNA northern

Ryd _{H1} for	<u>GAAATTAATACGACTCACTATAGGCTTCCGATGTAGACCCGTATTCTT</u> CGCCTGTACCTGCCAGGGTTTTAGTACAGGCGTTTTCTT	Ryd _{H1} transcription
Ryd _{H1} rev	AAGAAAACGCCTGTACTAAAACCCTGGGCAGGTACAGGCGAAGAAT ACGGGTCTACATCGGAAGCCTATAGTGAGTCGTATTAATTC	Ryd _{H1} transcription
Ryd _{H2} for	<u>GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT</u> CGCCTGTACCACGGGTCGGTTTTAGTACAGGCGTTTTCTT	Ryd _{H2} transcription
Ryd _{H2} rev	AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCGAAGAATT GCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTC	Ryd _{H2} transcription
Ryd _{H3} for	<u>GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT</u> CGCCTGTACCTGCCAGGGTTTTAGTACAGGCGTTTTCTT	Ryd _{H3} transcription
Ryd _{H3} rev	AAGAAAACGCCTGTACCAAACCCTGGGCAGGTACAGGCGAAGAATT GCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTC	Ryd _{H3} transcription
RydCPCRQ1	AAGAAAACGCCTGTACTAA	Real-Time PCR
RydCPCRQ2	CTTCCGATGTAGACCCGTA	Real-Time PCR
tmRNAPCRQ1	GGCAAGCGAATGTAAAGACTGA	Real-Time PCR
tmRNAPCRQ2	CCGCGTCCGAAATTCCTA	Real-Time PCR
csgDPCRQ1	CACGGAATCAGCCCTCCTTA	Real-Time PCR
csgDPCRQ2	GCCGATACGCAGCTTATTCAG	Real-Time PCR