

**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.**

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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.

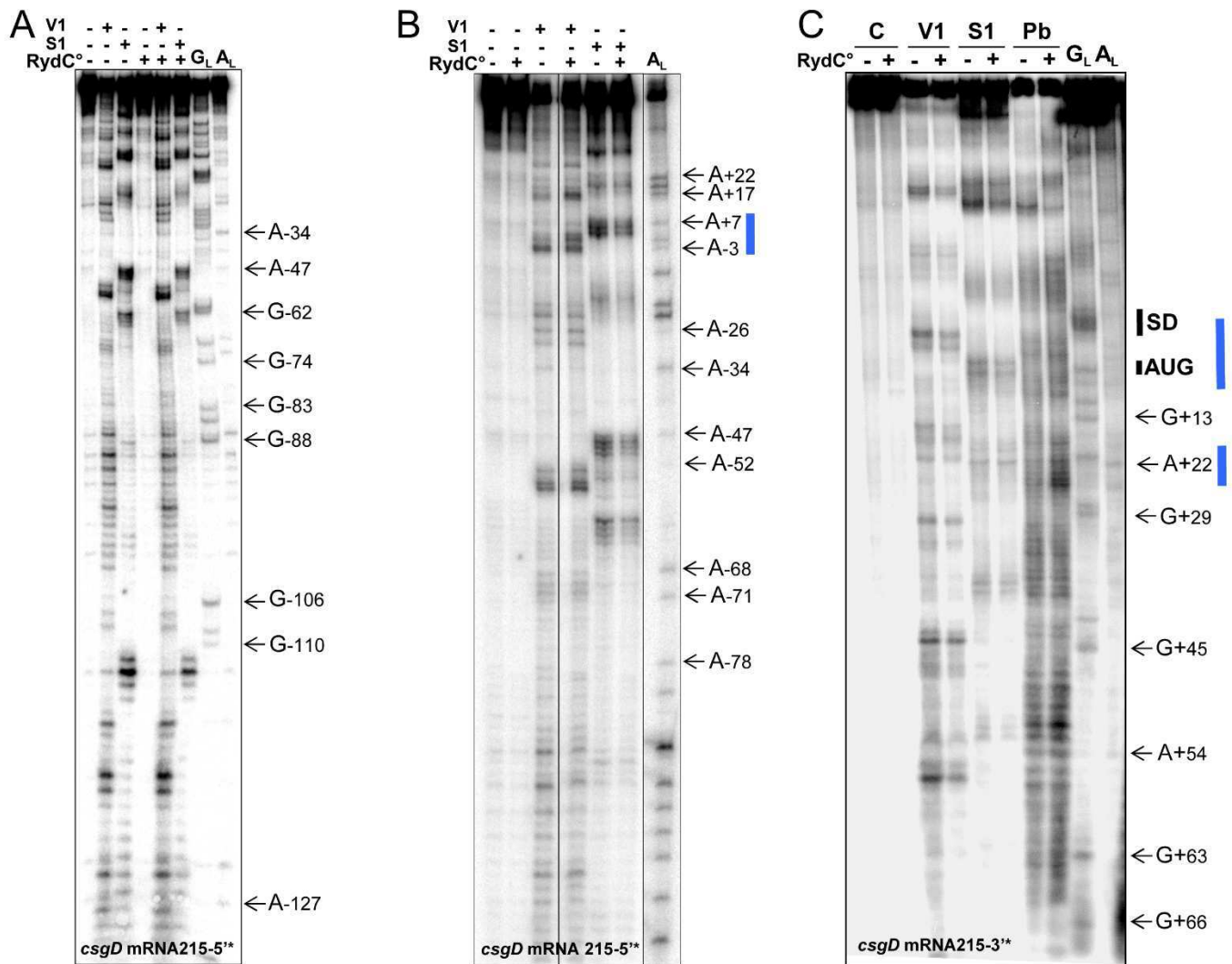
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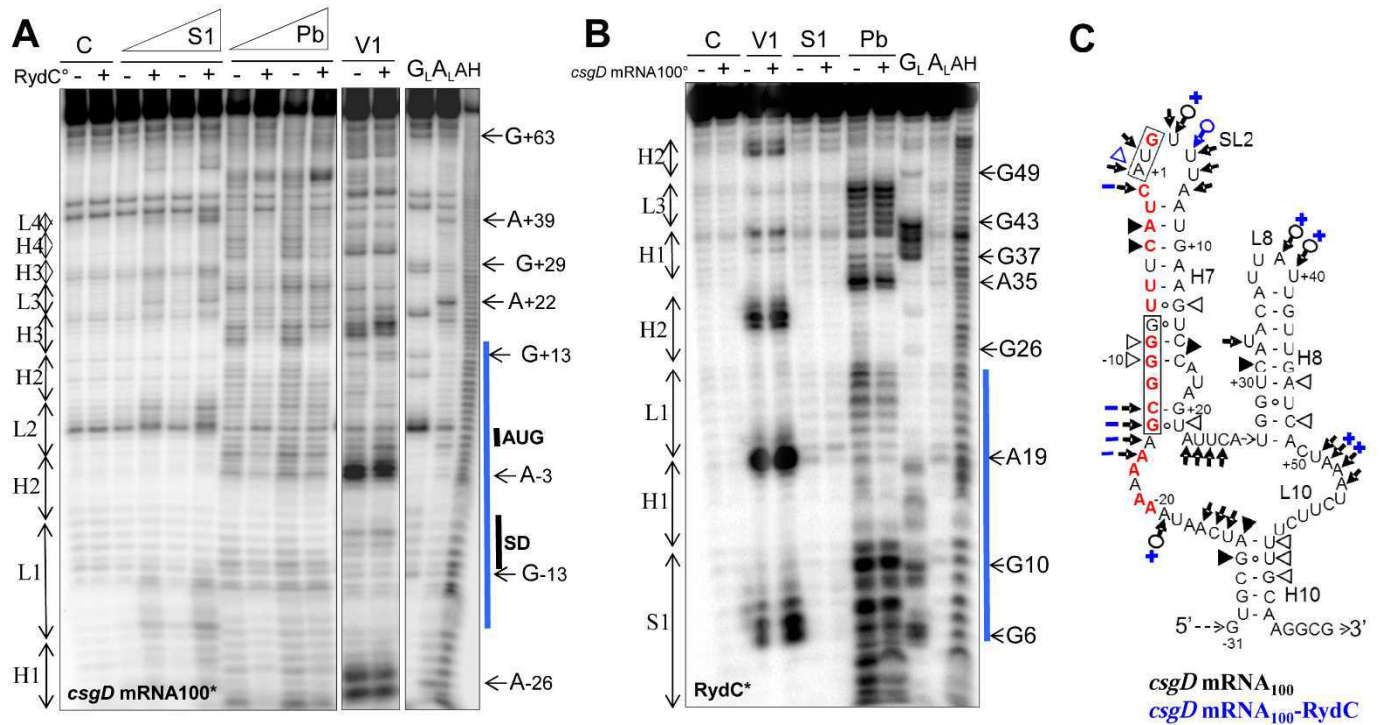
\*Corresponding author: [bfelden@univ-rennes1.fr](mailto:bfelden@univ-rennes1.fr)

*Supplemental Material*

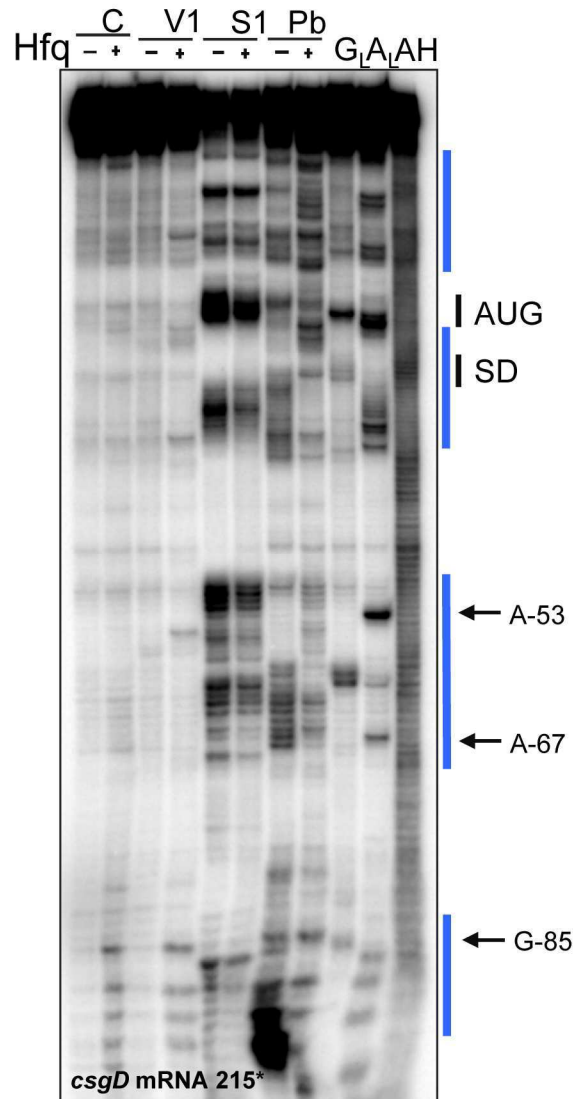




**Figure S2. Structural analysis of the conformational changes in *csgD* mRNA<sub>215</sub> induced by complex formation with RydC.** Autoradiograms of the cleavage products of 5'- (A, B) or 3'-labelled (C) *csgD* mRNA<sub>215</sub> by RNases V<sub>1</sub> (5.10<sup>-5</sup> unit) and nuclease S<sub>1</sub> (2 units) in the presence or absence of unlabelled RydC at a 1:100 molar ratio. Lanes G<sub>L</sub>, RNase T<sub>1</sub> hydrolysis ladder; lanes A<sub>L</sub>, RNase U<sub>2</sub> 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* mRNA<sub>100</sub> induced by complex formation with *csgD* mRNA<sub>100</sub> and RydC, respectively.** **A, B.** Autoradiograms of cleavage products of 5'-labelled *csgD* mRNA<sub>100</sub> (100 nts-long) (A) or 5'-labelled RydC (B) by RNases V<sub>1</sub> (5.10<sup>-5</sup> unit), nuclease S<sub>1</sub> (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* mRNA<sub>100</sub> (B) at 1:100 molar ratios. The *csgD* mRNA<sub>100</sub> or RydC structural domains are indicated on the left sides of each panel. Upon complex formation, the conformational changes of *csgD* mRNA<sub>100</sub> or RydC are highlighted by vertical blue bars. **C.** Secondary structure of the *csgD* mRNA<sub>100</sub> inferred from the probing results, which support the proposed model. Triangles are V<sub>1</sub> cuts; arrows capped by a circle are S<sub>1</sub> 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* mRNA<sub>100</sub> upon RydC complex formation are in blue.



**Figure S4. Structural analysis of the conformational changes of *csgD* mRNA<sub>215</sub> induced by complex formation with Hfq.** Autoradiograms of the cleavage products of 5' *csgD* mRNA<sub>215</sub> by RNases V<sub>1</sub> ( $15 \cdot 10^{-5}$  unit), nuclease S<sub>1</sub> (0.5 units), and lead acetate (1 mM) in the presence or absence of Hfq at a 1:20 molar ratio. Lanes G<sub>L</sub>, RNase T<sub>1</sub> hydrolysis ladder; lanes A<sub>L</sub>, RNase U<sub>2</sub> 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 $\Delta$ rydC                | Z1(lacR tetR SpR) $\Delta$ 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 <sub>215</sub> transcription toeprint<br><i>csgD</i> mRNA <sub>100</sub> transcription,<br><i>csgD</i> mRNA <sub>115</sub> transcription |
| csgD215for             | <u>TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTT</u><br>ACCC           | <i>csgD</i> mRNA <sub>215</sub> transcription   |
| csgD100for             | <u>TAATACGACTCACTATAGGGTGCATCAATAAAAAAAGCGGGG</u><br>TTTCAT          | <i>csgD</i> mRNA <sub>100</sub> transcription   |
| csgD503                | TTGCAACCCTTAATTGACAACACGTTCTTGAT                                     | <i>csgD</i> mRNA <sub>503</sub> transcription   |
| csgD $\Delta$ 5'UTRfor | <u>TAATACGACTCACTATAGGTTTAATGAAGTCCATAGTA</u>                        | <i>csgD</i> mRNA $\Delta$ 5'UTR   |
| csgD115rev             | ACCTGACAGCTGCCTCTAAAA  | <i>csgD</i> mRNA <sub>115</sub> transcription   |
| csgDnorth              | CAATGTCGCGGTACGGGTAATCTTCAGGCGTATTTAGCAA                             | <i>csgD</i> mRNA northern   |
| RydCfor                | <u>CGGGATCCTAATACGACTCACTATAGGGCTTCCGATGTAGACCC</u><br>GTT           | RydC transcription  |
| RydCrev                | AAGAAAACGCCTGTACTAAAAC   | RydC transcription  |
| RydCnorth              | ACCGACCCGTGGTACAGGCG   | RydC northern   |
| RydC $\Delta$ 5'for    | <u>TAATACGACTCACTATAGGCCTGTACCACGGGTCGGTTTTAGTA</u><br>CAGGCGTTTTCTT | RydC transcription  |
| RydC $\Delta$ 5'rev    | AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCCTAT<br>AGTGAGTCGTATTA        | RydC $\Delta$ 5' transcription  |
| tmRNAnorth             | GTTTTAACGCTTCAACCCCA   | tmRNA northern  |
| 5Snorth                | CTTCTGAGTTCGGCATGGGC   | 5S rRNA northern  |
| csgBAnorth             | AACTGCAGCACCGTTGCCACCACCGAACTGTTTAACCGTCATTT<br>AAT                  | <i>csgBA</i> mRNA northern  |

|                       |   |                                    |
|-----------------------|---|------------------------------------|
| Ryd <sub>H1</sub> for | <u>GAAATTAATACGACTCACTATAGGCTTCCGATGTAGACCCGTATTCTT</u><br>CGCCTGTACCTGCCAGGGTTTTAGTACAGGCGTTTTCTT  | Ryd <sub>H1</sub><br>transcription |
| Ryd <sub>H1</sub> rev | AAGAAAACGCCTGTACTAAAACCCTGGGCAGGTACAGGCGAAGAAT<br>ACGGGTCTACATCGGAAGCCTATAGTGAGTCGTATTAATTC         | Ryd <sub>H1</sub><br>transcription |
| Ryd <sub>H2</sub> for | <u>GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT</u><br>CGCCTGTACCACGGGTCGGTTTTAGTACAGGCGTTTTCTT | Ryd <sub>H2</sub><br>transcription |
| Ryd <sub>H2</sub> rev | AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCGAAGAATT<br>GCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTC          | Ryd <sub>H2</sub><br>transcription |
| Ryd <sub>H3</sub> for | <u>GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT</u><br>CGCCTGTACCTGCCAGGGTTTTAGTACAGGCGTTTTCTT  | Ryd <sub>H3</sub><br>transcription |
| Ryd <sub>H3</sub> rev | AAGAAAACGCCTGTACCAAACCCTGGGCAGGTACAGGCGAAGAATT<br>GCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTC           | Ryd <sub>H3</sub><br>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                      |