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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.

Valérie Bordeau and Brice Felden*

Université de Rennes I, Inserm U835-UPRES EA2311, Biochimie Pharmaceutique, 2 avenue du Prof. Léon Bernard 35043 Rennes, France.

*Corresponding author: bfelden@univ-rennes1.fr

Supplemental Material
Figure S1. Complex formation between RydC and two csgD mRNA fragments of different lengths. csgD mRNA\textsubscript{100} and csgD mRNA\textsubscript{215} 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 mRNA\textsubscript{215} and csgD mRNA\textsubscript{100} (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 mRNA_{215} induced by complex formation with RydC. Autoradiograms of the cleavage products of 5’- (A, B) or 3’-labelled (C) csgD mRNA_{215} by RNases V_{1} (5.10^{-5} unit) and nuclease S_{1} (2 units) in the presence or absence of unlabelled RydC at a 1:100 molar ratio. Lanes G_{L}, RNase T_{1} hydrolysis ladder; lanes A_{L}, RNase U_{2} 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\textsubscript{100} induced by complex formation with csgD mRNA\textsubscript{100} and RydC, respectively. A, B. Autoradiograms of cleavage products of 5’-labelled csgD mRNA\textsubscript{100} (100 nts-long) (A) or 5’-labelled RydC (B) by RNases V\textsubscript{1} (5 \times 10^{-5} unit), nuclease S\textsubscript{1} (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\textsubscript{100} (B) at 1:100 molar ratios. The csgD mRNA\textsubscript{100} or RydC structural domains are indicated on the left sides of each panel. Upon complex formation, the conformational changes of csgD mRNA\textsubscript{100} or RydC are highlighted by vertical blue bars. C. Secondary structure of the csgD mRNA\textsubscript{100} inferred from the probing results, which support the proposed model. Triangles are V\textsubscript{1} cuts; arrows capped by a circle are S\textsubscript{1} 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\textsubscript{100} upon RydC complex formation are in blue.
Figure S4. Structural analysis of the conformational changes of csgD mRNA induced by complex formation with Hfq. Autoradiograms of the cleavage products of 5’csgDmRNA215 by RNases V₁ (15.10⁻⁵ 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 G₁, RNase T₁ hydrolysis ladder; lanes A₁, 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 |
|-------------------------------|--------------------------------------------------|-------------------|
| *E. coli* MG1655Z1            | Z1(lacR tetR SpR)                                | (4)               |
| *E. coli* MG1655Z1 ΔrydC      | Z1(lacR tetR SpR) ΔRNA1114::Cm                    | (4)               |
| *E. coli* MG1655Z1 pUC18      | MG1655Z1 + pUC18                                 | (4)               |
| *E. coli* MG1655Z1 pUC18-rydC | MG1655Z1 + pUC18-RNA1114                         | (4)               |
| *S. enterica* subsp. *bongori*+pUC18 | *Salmonella enterica subsp. bongori* + pUC18       | This study        |
| *S. enterica* subsp. *bongori*+pUC18-rydC | *Salmonella enterica subsp. bongori* + pUC18-RNA1114 | This study        |
| *S. sonnei*+pUC18            | *Shigella sonnei* + pUC18                        | This study        |
| *S. sonnei*+pUC18-rydC       | *Shigella sonnei* + pUC18-RNA1114                | This study        |

Table S2. DNA oligodeoxyribonucleotides used in this study.

| Names                      | Sequences (5’→3’) | Purposes                                      |
|----------------------------|-------------------|-----------------------------------------------|
| csgD215rev                 | CGCCTGCAAAGAAGATTTAGT | csgD mRNA<sub>215</sub> transcription        |
|                            |                   | csgD mRNA<sub>100</sub> transcription        |
|                            |                   | csgD mRNA<sub>115</sub> transcription        |
| csgD215for                 | TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTTTACCC | csgD mRNA<sub>215</sub> transcription        |
| csgD100for                 | TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTTTTTACCC | csgD mRNA<sub>100</sub> transcription        |
|                            |                   | csgD mRNA<sub>115</sub> transcription        |
| csgD503                    | TTGCAACCCTTAATTGACACACGGTTCTTGAT                 | csgD mRNA<sub>503</sub> transcription        |
| csgDΔ5’UTRfor              | TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTTTTTACCC | csgD mRNA<sub>Δ5’UTR</sub>                  |
| csgD115rev                 | ACCTGACAGCTGCTCCTCTAAA | csgD mRNA<sub>115</sub> transcription        |
| csgDnorth                  | CAATGTCCGGTGACGGGTAATCTTCAGGGCGTTTTAGCAA         | csgD mRNA<sub>northern</sub>                 |
| RydCfor                    | CCGGATCTCTAAATACGACTCCTAGGGCCTCGATGACCGTCGTTTTAGTTT | RydC transcription                           |
| RydCrev                    | AAGAAAAACGCCTGACTA AAAA | RydC transcription                           |
| RydCnorth                  | ACCGACCCGTTGGTACAGGCC | RydC<sub>northern</sub>                       |
| RydC<sub>Δ5’</sub>for      | TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATAGTA CAGGGCGTTTTTTCTT | RydC<sub>Δ5’</sub> transcription           |
| RydC<sub>Δ5’</sub>rev      | AAGAAAAACGCCTGACTA AAAA ACCGACCCCGTGTTACAGGGCCTATTAGTATA GTGAGTCGTTATTA | RydC<sub>Δ5’</sub> transcription           |
| tmRNA<sub>northern</sub>   | GTTTTAACGCTTCAACCCCA | tmRNA<sub>northern</sub>                      |
| 5Snorthern                 | CTTCTGAGTGGCAGCTGCCC | 5S rRNA<sub>northern</sub>                   |
| csgBA<sub>northern</sub>   | AACTGCAAGCACCCTGCTGACCACCAACCAGAAGCTATTAAACCGTCATT | csgBA<sub>northern</sub>                   |
| RydC_{H1}for | GAAATTAATACGACTCACTATAGGCTCCGATGTAGACCCTGATTCTTT CGCCTGTACCCTCCAGGGTTTTAGTACAGGCGTTTTCTT | RydC_{H1} transcription |
| --- | --- | --- |
| RydC_{H1}rev | AAGAAAACGCCTGTACTAAAAACCCCTGGCCAGGTACAGGCAAAGAAAT ACGGGTCTACATCGGAAGCCTATAGTGAGTCGTATTAATTTCC | RydC_{H1} transcription |
| RydC_{H2}for | GAAATTAATACGACTCCTAGGCTTCCGATGTACTGGGCAATTCTT CGCCTGTACCACGGGTCGGTTTTAGTACAGGCGTTTTCTT | RydC_{H2} transcription |
| RydC_{H2}rev | AAGAAAACGCTGTACTAAAAACCGACCCTGGGTACAGGCGAAAGAATT GCCCTGTACATCGGAAGCCTATAGTGAGTCGTATTAATTTCC | RydC_{H2} transcription |
| RydC_{H3}for | GAAATTAATACGACTCCTAGGCTTCCGATGTACTGGGCAATTCTT CGCCTGTACCCTCCAGGGTTTTAGTACAGGCGTTTTCTT | RydC_{H3} transcription |
| RydC_{H3}rev | AAGAAAACGCCTGTACTAAAAACCGACCCTGGGTACAGGCGAAAGAATT GCCCTGTACATCGGAAGCCTATAGTGAGTCGTATTAATTTCC | RydC_{H3} transcription |
| RydCPCRQ1 | AAGAAAACGCCTGTACTAA | Real-Time PCR |
| RydCPCRQ2 | CTTCCGATGTAGACCCGTA | Real-Time PCR |
| tmRNAPCRQ1 | GGCAAGCGAATGTAAAGACTGA | Real-Time PCR |
| tmRNAPCRQ2 | CCGCGTCCGAAATCTCTTA | Real-Time PCR |
| csgDPCRQ1 | CACCGGAATCAGCCCTCCTTA | Real-Time PCR |
| csgDPCRQ2 | GCCGATACGCAGCTATTTCAG | Real-Time PCR |