The *Escherichia coli ribB* riboswitch senses flavin mononucleotide within a defined transcriptional window

Sébastien H. Eschbach[#], Elsa D. M. Hien, Tithi Ghosh[@], Anne-Marie Lamontagne[†] and Daniel A. Lafontaine*

Department of Biology, Faculty of Science, RNA Group, Université de Sherbrooke, Sherbrooke, Quebec, Canada, J1K 2R1.

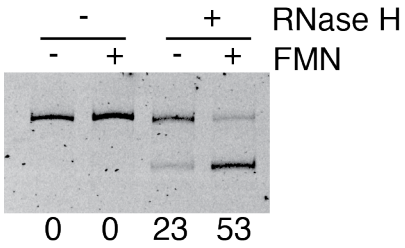
[#]Present address: New World River Expeditions, Argenteuil, Quebec, Canada

[@]Present address: Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada.

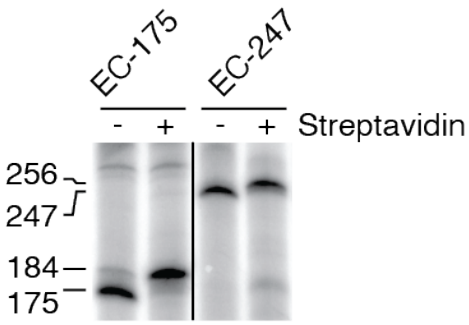
[†]Present address: Charles River Laboratories, Sherbrooke, Quebec, Canada.

*Corresponding author. E-mail: daniel.lafontaine@usherbrooke.ca

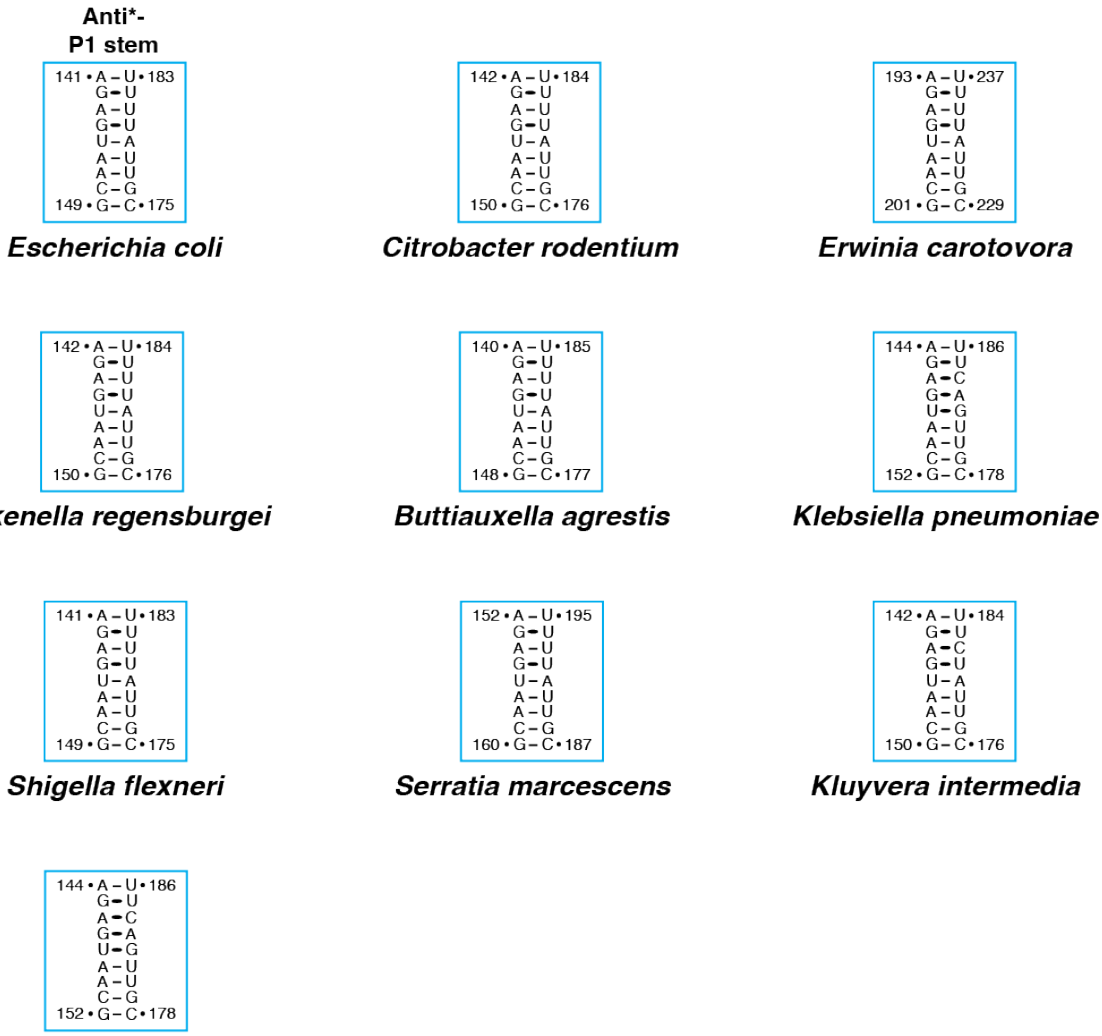
Keywords: Riboswitch genetic regulation, nascent RNA, transcription, FMN.



Supplemental Figure S1. RNase H cleavage assays of the *ribB* riboswitch.
RNase H assays were performed on the FL-282 construct using the probe 184. The experiments were done using Cy3-labelled transcripts. While no cleavage is observed in the absence of RNase H, the transcripts are cleaved with RNase H in the absence and presence of FMN. The percentages of cleavage are shown below the gel.

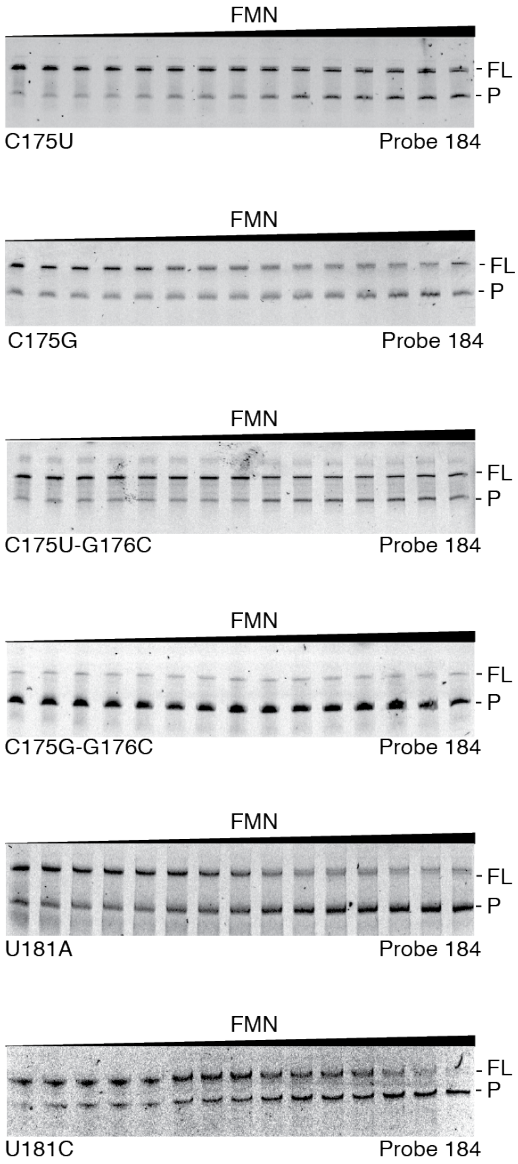


Supplemental Figure S2. Transcription elongation complexes are formed using a biotin-streptavidin roadblock.
Formation of stable transcription elongation complex in the presence of biotin-streptavidin roadblocks. Transcription reactions were performed without (-) or with (+) streptavidin. The formation of the expected transcripts (175 nt and 247 nt) is obtained in the presence of streptavidin. Transcriptions performed in the absence of streptavidin result in RNA molecules being extended by 9 nt, which corresponds to 184 and 256 nt, respectively (Chauvier et al. 2017).



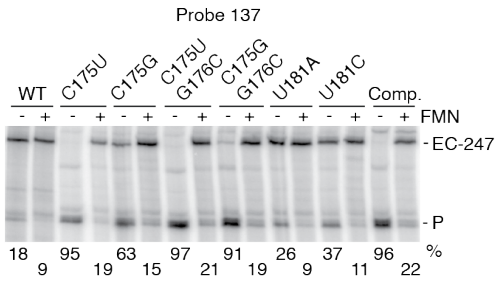
Supplemental Figure S3. Predicted secondary structures for the anti*-P1 stem in different bacterial species.

Bacteria were selected within the Enterobacterales order using Rfam (Griffiths-Jones et al. 2003). In the case of *Klebsiella pneumonia*, *Kluyvera intermedia* and *Raoultella ornithinolytica*, a C-A base pair was allowed in the stem.



Supplemental Figure S4. RNase H probing assays of mutants of the anti*-P1 stem.

RNase H probing assays performed on full-length nascent transcripts using the probe 184. Cleavage assays were performed using a range of 2 nM to 10 μM FMN. Full-length (FL) and cleaved products (P) are indicated on the right of the gels. The fitting analysis and K_{switch} values are shown in Fig. 5B,C.



Supplemental Figure S5. RNase H probing assays obtained on EC-247 *ribB* nascent transcripts with the probe 137.

Experiments were performed without (-) and with (+) 100 µM FMN. The WT and the mutants used are indicated at the top. The full-length EC-247 transcripts (EC-247) and cleaved products (P) are indicated on the right of the gel.

Supplemental Table S1. Summary of strains or plasmids used in this study.

	Strains	
Strain name	**Characteristics**	**References**
EM1055	WT strain- MG1655 *ΔlacX74*	Caron *et al.*, 2012 (Caron et al. 2012)
	Plasmids	
Plasmid name	**Characteristics**	**References**
pUA66/pbpG-GFP	pUC101 ori, KanR	Alon collection (Zaslaver et al. 2006)
pTGX/FL-282	pUA66/placUV5-FL-282-fluo	This study
pTGX/FL-282-C175U	pUA66/placUV5-FL-282-fluo C175U	This study
pTGX/FL-282-C175G	pUA66/placUV5-FL-282-fluo C175G	This study
pTGX/FL-282-C175U-G176C	pUA66/placUV5-FL-282-fluo C175U-G176C	This study
pTGX/FL-282-C175G-G176C	pUA66/placUV5-FL-282-fluo C175G-G176C	This study
pTGX/FL-282-U181A	pUA66/placUV5-FL-282-fluo U181A	This study
pTGX/FL-282-U181C	pUA66/placUV5-FL-282-fluo U181C	This study

Supplemental Table S2. PCR constructs used for *in vitro assays.*

***In vitro* transcription assays**	
Constructs	**PCR strategy**
Radioactivity-based detection	
FL-282	RibL1 + FMN076 (genomic DNA as template)
EC-175	RibL1 + FMN120 (genomic DNA as template)
EC-247	RibL1 + FMN122 (genomic DNA as template)
EC-247 C175U	PCR1: RibL1 + FMN237 (genomic DNA as template) PCR2: FMN236 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 C175G	PCR1: RibL1 + FMN235 (genomic DNA as template) PCR2: FMN234 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 C175U:G176C	PCR1: RibL1 + FMN241 (genomic DNA as template) PCR2: FMN240 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 C175G:G176C	PCR1: RibL1 + FMN239 (genomic DNA as template) PCR2: FMN238 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 U181A	PCR1: RibL1 + FMN245 (genomic DNA as template) PCR2: FMN244 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 U181C	PCR1: RibL1 + FMN247 (genomic DNA as template) PCR2: FMN246 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
EC-247 Comp	PCR1: RibL1 + FMN243 (genomic DNA as template) PCR2: FMN242 + FMN122 (genomic DNA as template) PCR3: RibL1 + FMN122 (PCR1 and PCR2 as templates)
Fluorescence-based detection	
Linear vector backbone	Fwd RBS-GFP + Rev backbone-pUA66-lacUV5overhang (pUA66/pbpG-GFP plasmid as template)
FL-282-fluo WT	Fwd lacUV5-*ribB* + Rev *ribB*-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo C175U	PCR1: Fwd lacUV5-ribB + Rev ribB-C175U (genomic DNA as template) PCR2: Fwd ribB-C175U + Rev ribB-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo C175G	PCR1: Fwd lacUV5-ribB + Rev ribB-C175G (genomic DNA as template) PCR2: Fwd ribB-C175G + Rev ribB-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo C175U-G176C	PCR1: Fwd lacUV5-ribB + Rev ribB-C175U-G176C (genomic DNA as template) PCR2: Fwd ribB-C175U-G176C + Rev ribB-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo C175G-G176C	PCR1: Fwd lacUV5-ribB + Rev ribB-C175G-G176C (genomic DNA as template) PCR2: Fwd ribB-C175G-G176C + Rev ribB-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo U181A	PCR1: Fwd lacUV5-ribB + Rev ribB-U181A (genomic DNA as template) PCR2: Fwd ribB-U181A + Rev ribB-6cd-RBS-GFP (genomic DNA as template)
FL-282-fluo U181C	PCR1: Fwd lacUV5-ribB + Rev ribB-U181C (genomic DNA as template) PCR2: Fwd ribB-U181C + Rev ribB-6cd-RBS-GFP (genomic DNA as template)

Supplemental Table S3. Summary of oligonucleotides used in this study.

Oligonucleotides	Sequences 5' to 3'
RNase H assays	
FMN014 (RNase H 137)	GTTACTCTCTCC
FMN126 (RNase H 180)	ATAGCCAAAA
FMN127 (RNase H 181)	TATAGCCAAA
FMN128 (RNase H 182)	ATATAGCCAA
FMN129 (RNase H 183)	CATATAGCCA
FMN130 (RNase H 184)	GCATATAGCC
FMN131 (RNase H 185)	GGCATATAGC
FMN132 (RNase H 186)	CGGCATATAG
FMN133 (RNase H 187)	GCGGCATATA
FMN134 (RNase H 188)	GGCGGCATAT
FMN135 (RNase H 189)	TGGCGGCATA
FMN136 (RNase H 190)	GTGGCGGCAT
FMN137 (RNase H 191)	AGTGGCGGCA
FMN138 (RNase H 192)	GAGTGGCGGC
FMN139 (RNase H 193)	GGAGTGGCGG
FMN140 (RNase H 194)	AGGAGTGGCG
FMN141 (RNase H 195)	TAGGAGTGGC
FMN142 (RNase H 196)	TTAGGAGTGG
FMN143 (RNase H 197)	CTTAGGAGTG
FMN144 (RNase H 198)	TCTTAGGAGT
FMN145 (RNase H 199)	GTCTTAGGAG
FMN146 (RNase H 200)	AGTCTTAGGA
FMN147 (RNase H 201)	CAGTCTTAGG
FMN148 (RNase H 202)	GCAGTCTTAG
FMN149 (RNase H 203)	GGCAGTCTTA
FMN150 (RNase H 204)	GGGCAGTCTT
FMN151 (RNase H 205)	AGGGCAGTCT
FMN152 (RNase H 206)	CAGGGCAGTC
FMN153 (RNase H 207)	TCAGGGCAGT
FMN154 (RNase H 208)	ATCAGGGCAG
FMN155 (RNase H 209)	AATCAGGGCA
FMN156 (RNase H 210)	GAATCAGGGC
FMN157 (RNase H 211)	AGAATCAGGG
FMN158 (RNase H 212)	CAGAATCAGG
FMN159 (RNase H 213)	CCAGAATCAG
FMN160 (RNase H 214)	ACCAGAATCA
FMN162 (RNase H 215)	TACCAGAATC
FMN163 (RNase H 216)	TTACCAGAAT
FMN164 (RNase H 217)	GTTACCAGAA
FMN165 (RNase H 218)	GGTTACCAGA
FMN166 (RNase H 219)	TGGTTACCAG
FMN167 (RNase H 220)	ATGGTTACCA
FMN168 (RNase H 221)	TATGGTTACC
FMN169 (RNase H 222)	TTATGGTTAC
FMN170 (RNase H 223)	ATTATGGTTA
FMN171 (RNase H 224)	AATTATGGTT
FMN172 (RNase H 225)	AAATTATGGT
FMN173 (RNase H 226)	AAAATTATGG
FMN174 (RNase H 227)	TAAAATTATG
PCR templates	
RibL1 (*ribB* natural promoter)	TTCCCTGTACTGATAGGTGTTG
FMN076 (Rev. primer to obtain 30 nt in the ORF)	ACCAAAAGAGGAAAGTAGCGTC
FMN120 (Rev. primer to obtain EC-175)	/5Biosg/CAAAATAACGTGAGCGGGTCCATGC
FMN122 (Rev. primer to obtain EC-247)	/5Biosg/TCATGGTAAAAAAACCTCACTAAAA
FMN234 (For. primer for C175G mutant)	GCATGGACCCGCTCAGGTTATTTTGGCTATATGCCGCCA
FMN235 (Rev. primer for C175G mutant)	TGGCGGCATATAGCCAAAATAACCTGAGCGGGTCCATGC
FMN236 (For. primer for C175U mutant)	GCATGGACCCGCTCAUGTTATTTTGGCTATATGCCGCCA
FMN237 (Rev. primer for C175U mutant)	TGGCGGCATATAGCCAAAATAACATGAGCGGGTCCATGC
FMN238 (For. primer for C175G-G176C mutant)	GCATGGACCCGCTCAGCTTATTTTGGCTATATGCCGCCA
FMN239 (Rev. primer for C175G-G176C mutant)	TGGCGGCATATAGCCAAAATAAGCTGAGCGGGTCCATGC
FMN240 (For. primer for C175U-G176C mutant)	GCATGGACCCGCTCAUCTTATTTTGGCTATATGCCGCCA

FMN241 (Rev. primer for C175U-G176C mutant)	TGGCGGCATATAGCCAAAATAAGATGAGCGGGTCCATGC
FMN242 (For. primer for Comp mutant)	GCATGGACCCGCTCAGCAATAAAAGGCTATATGCCGCCA
FMN243 (Rev. primer for Comp mutant)	TGGCGGCATATAGCCTTTTATTGCTGAGCGGGTCCATGC
FMN244 (For. primer for U181A mutant)	GCATGGACCCGCTCACGTTATATTGGCTATATGCCGCCA
FMN245 (Rev. primer for U181A mutant)	TGGCGGCATATAGCCAATATAACGTGAGCGGGTCCATGC
FMN246 (For. primer for U181C mutant)	GCATGGACCCGCTCACGTTATCTTGGCTATATGCCGCCA
FMN247 (Rev. primer for U181C mutant)	TGGCGGCATATAGCCAAGATAACGTGAGCGGGTCCATGC
Fluorescence-based detection	
Fwd RBS-GFP	GGATCCTCTAGATTTAAGAAGGAGATATACATATGAG
Rev backbone-pUA66-lacUV5overhang	GCATAAAGTGTAAAGCCTGGGGTGCCCGTGAAGACGAAAGG GCCTCGTG
Fwd lacUV5-ribB (For. primer for ribB-WT)	GGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTAT AATGTGTGGGCTTATTCTCAGGGCGGGGC
Rev *ribB*-6cd-RBS-GFP (Rev. primer for ribB-WT)	CTCCTTCTTAAATCTAGAGGATCCAAGTAGCGTCTGATTCATG GTAAAAAAACCTCAC
Rev ribB C175U	GCATATAGCCAAAATAACATGAGCGGGTCC
Fwd *ribB* C175U	GGACCCGCTCATGTTATTTTGGCTATATGC
Rev *ribB* C175G	GCCAAAATAACCTGAGCGGGTCC
Fwd *ribB* C175G	GGACCCGCTCAGGTTATTTTGGC
Rev *ribB* C175U-G176C	CATGGACCCGCTCATCTTATTTTGGCTATATG
Fwd *ribB* C175U-G176C	CATGGACCCGCTCATCTTATTTTGGCTATATG
Rev *ribB* C175G-G176C	GGACCCGCTCAGCTTATTTTGGC
Fwd *ribB* C175G-G176C	GGACCCGCTCAGCTTATTTTGGC
Rev *ribB* U181A	CGGCATATAGCCAATATAACGTGAGCGG
Fwd *ribB* U181A	CCGCTCACGTTATATTGGCTATATGCCG
Rev *ribB* U181C	GGCATATAGCCAAGATAACGTGAGCGG
Fwd *ribB* U181C	CCGCTCACGTTATCTTGGCTATATGCC

Supplemental References

Caron M-P, Bastet L, Lussier A, Simoneau-Roy M, Massé E, Lafontaine DA. 2012. Dual-acting riboswitch control of translation initiation and mRNA decay. *Proceedings of the National Academy of Sciences* **109**: E3444-53.

Chauvier A, Picard-Jean F, Berger-Dancause J-C, Bastet L, Naghdi MR, Dubé A, Turcotte P, Perreault J, Lafontaine DA. 2017. Transcriptional pausing at the translation start site operates as a critical checkpoint for riboswitch regulation. *Nat Commun* **8**: 13892.

Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. 2003. Rfam: an RNA family database. *Nucleic Acids Res* **31**: 439–441.

Zaslaver A, Bren A, Ronen M, Itzkovitz S, Kikoin I, Shavit S, Liebermeister W, Surette MG, Alon U. 2006. A comprehensive library of fluorescent transcriptional reporters for *Escherichia coli*. *Nat Methods* **3**: 623–8.