Direct and Indirect Control of Rho-Dependent Transcription Termination by the *Escherichia coli lysC* Riboswitch

T. Ghosh[1], S. Jahangirnejad[1], A. Chauvier[1&], A.M. Stringer[2], A.P. Korepanov[4], J.P. Côté[1], J.T. Wade[2,3*] and D.A. Lafontaine[1*]

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

[2]Wadsworth Center, New York State Department of Health, Albany, NY 12208, USA.

[3]Department of Biomedical Sciences, University at Albany, Albany, NY 12201, USA.

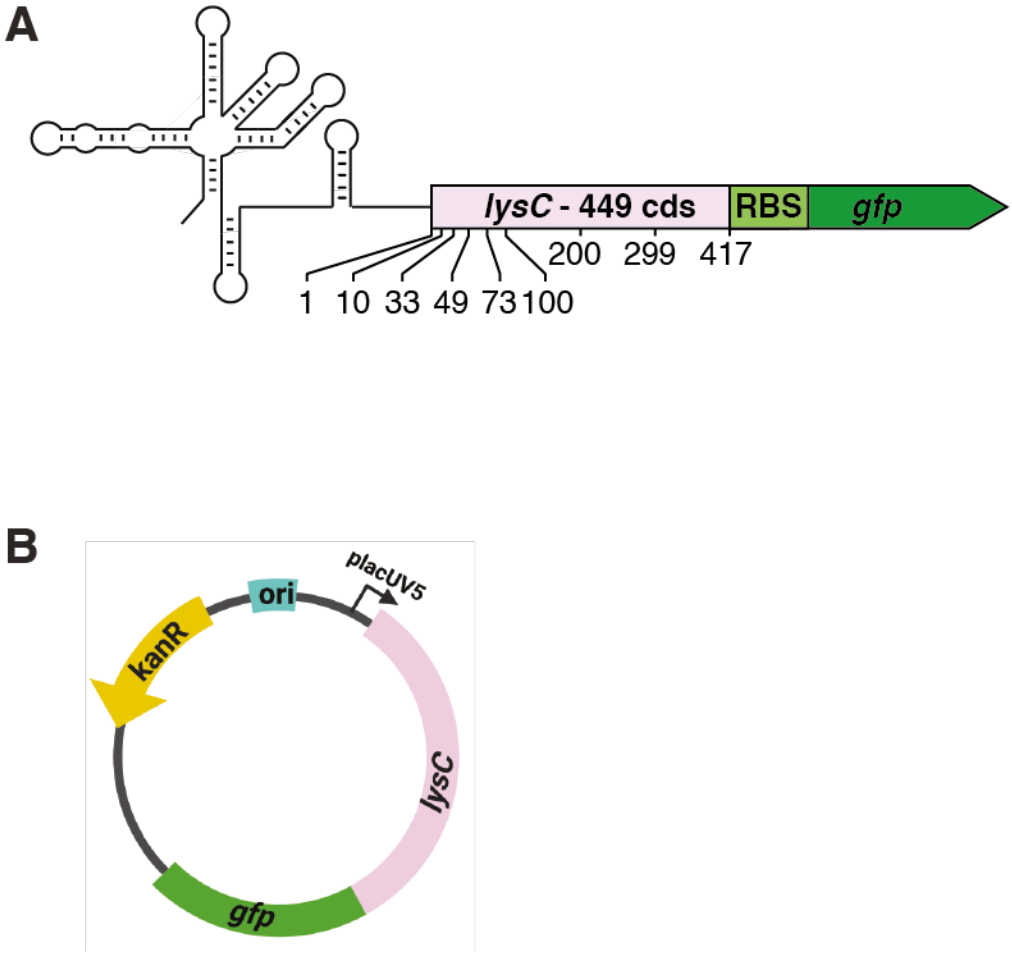
[4]Expression Génétique Microbienne, UMR8261 CNRS, Université Paris Cité, Institut de Biologie Physico-Chimique, 75005 Paris, France.

[&]Present address: Single Molecule Analysis Group, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109.

*Corresponding authors: joseph.wade@health.ny.gov and daniel.lafontaine@usherbrooke.ca

Short title: Direct and Indirect Control of the *lysC* Riboswitch

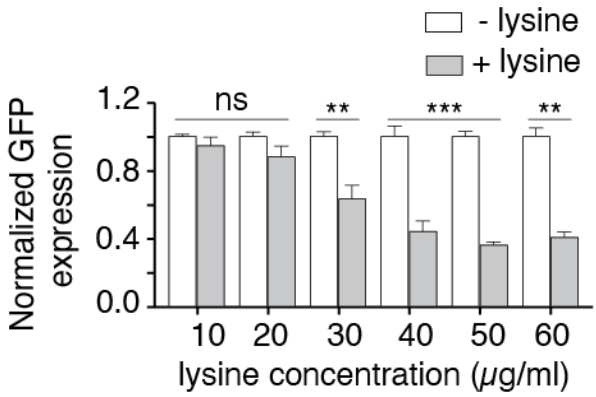
Keywords: Riboswitch, lysine, Rho transcription termination, reporter gene assays.



Supplemental Figure S1. Schematics of the constructs used in this study.

(*A*) Transcriptional *lysC-gfp* fusions used for reporter gene assays. The various lengths used in this study are shown. The presence of the ribosome binding site (RBS) of *gfp* allows the construct to report mRNA levels and not to be directly modulated by the translational regulation of the riboswitch.

(*B*) Schematic of the plasmid used to express the transcriptional *lysC-gfp* fusions.



Supplemental Figure S2. Expression of transcriptional *lysC-gfp* fusion performed in the absence and the presence of various concentrations of lysine.

The construct used in this experiment contained 417 codons of *lysC.* Values were normalized to the expression obtained without lysine for each construct. The average values of three independent experiments with SD are shown. Two and three asterisks indicate the p-value of <0.01 and <0.001, respectively, and NS stands for non-significant.

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

	Strains	
Strain name	**Characteristics**	**References**
EM1055	WT strain- MG1655 *Δlac*X74	Caron *et al.*, 2012 (Caron et al. 2012)
EM1377	EM1055 *rne-131 zce-726*::Tn*10*	Caron *et al.*, 2012 (Caron et al. 2012)
137AML	EM1055, placUV5 *lysC* 308 nt *lacZ* (transcriptional fusion)	This study
138AML	EM1377 *rne-131*, placUV5 *lysC* 308 nt *lacZ* (transcriptional fusion)	This study
CRB016	MG1655 *rho*-R66S *ilv-500*::Tn*10*	Bastet *et al.*, 2017 (Bastet et al. 2017)
OK580	MG1655 *Δlac*X74 *mal::lacI*^{q} *rho*-35::IS*1*	
OK752	MG1655 *Δlac*X74 *mal::lacI*^{q} *rho*-R66S *ilv-500*::Tn*10*	This study
OK756	MG1655 *Δlac*X74 *mal::lacI*^{q} *rho*-R66S	This study
	Plasmids	
Plasmid name	**Characteristics**	**References**
pBAD *lysC*-WT	pBAD24/*lysC* (arabinose inducible promoter)	Caron *et al.*, 2012 (Caron et al. 2012)
pBAD *lysC*-ΔSite1	pBAD24/*lysC*-ΔSite1 (arabinose inducible promoter)	Caron *et al.*, 2012 (Caron et al. 2012)
pUA66/pbpg-GFP	pUC101 ori, KanR	Alon collection (Zaslaver et al. 2006)
pTG268	pUA66/placUV5-RBS-GFP	This study
pTG289	pUA66/placUV5-*lysC* 1cd-RBS-GFP	This study
pTG273	pUA66/placUV5-*lysC* 10cd-RBS-GFP	This study
pTG290	pUA66/placUV5-*lysC* 30cd-RBS-GFP	This study
pTG274	pUA66/placUV5-*lysC* 50cd-RBS-GFP	This study
pTG275	pUA66/placUV5-*lysC* 75cd-RBS-GFP	This study
pTG257	pUA66/placUV5-*lysC* 100cd-RBS-GFP	This study
pTG276	pUA66/placUV5-*lysC* 200cd-RBS-GFP	This study
pTG277	pUA66/placUV5-*lysC* 300cd-RBS-GFP	This study
pTG278	pUA66/placUV5-*lysC* 400cd-RBS-GFP	This study
pTG377	pUA66/placUV5-*lysC* ΔSite1 1cd-RBS-GFP	

Supplemental Table S2. Summary of oligonucleotides used in this study.

Name	Sequence 5'-3'	Use
Fwd LacUV5-lysC	GGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATG TGTGGGTACTACCTGCGCTAGCGCA	*lysC*-GFP fusion construct, common forward
Rev lysC 100nt	GCACGTTGGCATCAGAAAGCACAATATC	PCR product for *in vitro* transcription, reverse
Probe lysC P1	CGCGCCTCTTCTGG	
Rev lysC 1cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCCATAACTACCTCGTGTCAGG GGATCC	pTG289 and pTG377 construct, reverse
Rev lysC 10cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCGCCAAATTTGGAGACAACAA TTTCAGACATAAC	pTG273 construct, reverse
Rev lysC 30cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCGCACGTTGGCATCAGAAAGC AC	pTG290 construct, reverse
Rev lysC 50cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCGACCAGCAGATTAGTGATAC CAGCAG	pTG274 construct, reverse
Rev lysC 75cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCGGCAAACTGGATGTTGCGGA TAGC	pTG275 construct, reverse
Rev lysC 100cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCTTCTGCCAGAACAGTAATGTT CTCCAGC	pTG257 construct, reverse
Rev lysC 200cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCCACGGCCAAGCGTCGTTGTA C	pTG276 construct, reverse
Rev lysC 300cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCGAACAGCGGCGGATTTTCAG TTTTATTG	pTG277 construct, reverse
Rev lysC 400cd-RBS-GFP	CTCCTTCTTAAATCTAGAGGATCCCATGCGAATGTTGAACGGTT CCAGTAC	pTG278 construct, reverse
Fwd RBS-GFP on pUA66	GGATCCTCTAGATTTAAGAAGGAGATATACATATGAG	pUA66 backbone amplification, forward
Rev backbone pUA66+ lacUV5 overhang	GCATAAAGTGTAAAGCCTGGGGTGCCCGTGAAGACGAAAGGGC CTCGTG	pUA66 backbone amplification, reverse
Fwd sequencing on pUA66	CTTCCCAACCTTACCAGAGGGCG	Plasmid sequencing
Rev sequencing on pUA66	GCGGCGGATTTGTCCTACTCAG	Plasmid sequencing
Fwd rho (AK149)	GTGGGTGCACTGTCTAAAGGTC	*rho* locus check, forward
Rev rho (AK151)	CATCTCGGTTACTTCTTCCGGAC	*rho* locus check, reverse
qPCR primers		
Fwd gyrA	CTAATCCGTGGCAGCTGGGC	*gyrA* amplification, forward
Rev gyrA	ATCACGCACGCCGAACTCTG	*gyrA* amplification, reverse
Fwd rpoZ	TGGCACGCGTAACTGTTCAGG	*rpoZ* amplification, forward
Rev rpoZ	CCGCCTACCTGCATCTGACG	*rpoZ* amplification, reverse
Fwd rssA	CGGTTGACCTGCAGCACGAT	*rssA* amplification, forward
Rev rssA	CGCATGCCACGGCAGAGAAT	*rssA* amplification, reverse
Fwd gfp	GGAGTTGTCCCAATTCTTGTTGAATTAGATGG	*gfp* amplification, forward
Rev gfp	GTGCAAATAAATTTAAGGGTAAGTTTTCCGTATGTTG	*gfp* amplification, reverse
Fwd lysC	CCATGAACCGCAGCGCTGATATTG	*lysC* amplification, forward
Rev lysC	CCTTCAGCTAAAGCGACCAGCAG	*lysC* amplification, reverse
ChIP-qPCR primers		
Fwd pBAD	CCATAGCATTTTTATCCA	pBAD promoter amplification, forward
Rev pBAD	AGAAGAGGCGCGTTG	pBAD promoter, reverse
Fwd C3	GCGCTGATATTGTGCTTT	*lysC* C3 amplification, forward
Rev C3	GCTGAAGGACTGGAACCT	*lysC* C3 amplification, reverse
Fwd C5	TACGCTGGTGTGCAATAA	*lysC* C5 amplification, forward
Rev C5	CTCTGCTCACTTTGCACA	*lysC* C5 amplification, reverse

Fwd C6	AGGTGGAAGAAGGTCTGG	*lysC* C6 amplification, forward
Rev C6	TATTCGGCGTACTGGAAC	*lysC* C6 amplification, reverse

Supplemental References

Bastet L, Chauvier A, Singh N, Lussier A, Lamontagne A-MM, Prévost K, Massé E, Wade JT, Lafontaine DA. 2017. Translational control and Rho-dependent transcription termination are intimately linked in riboswitch regulation. *Nucleic Acids Res* **45**: 7474–7486. http://www.ncbi.nlm.nih.gov/pubmed/28520932.

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. http://www.ncbi.nlm.nih.gov/pubmed/23169642.

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.