Supplemental Information

RNase HIII is important for Okazaki fragment Processing in *Bacillus subtilis*

Justin R. Randall[1], Taylor M. Nye[2], Katherine J. Wozniak[2] and Lyle A. Simmons[*]

Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109

*Corresponding author
LAS: Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109-1055, United States. Phone: (734) 647-2016, Fax: (734) 615-6337
E-mail: lasimm@umich.edu

[1]Present address: Department of Molecular Biosciences, University of Texas at Austin.

[2]These authors contributed equally

Running title: Okazaki fragment maturation

Table S1. Identification of genome variants in Δ*rnhC, rnhB::erm* resequencing

Gene	Mutation	Annotation	Frequency	Description
rpoC	A → C	Q148P	0.8	RNA-polymerase B` subunit
gutB	$A_{5 \rightarrow 4}$	Coding (28/1134nt)	1	Sorbitol dehydrogenase
coaA	G → T	A70D	1	Pantothenate kinase
rnhC →/← *pheT*	Δ1 bp	Intergenic (+10/+26)	1	Intergenic
yeaD	T → C	S194S	1	Unknown
yqxC	T → G	N135T	0.2	Putative rRNA methyltransferase
rsiX	G → A	T303T	0.2	Sigma factor X

Table S2. List of strains

Strain	Genotype	Citation
JRR1	*E. coli* BL21$_{DE3}$	
JRR2	*E. coli* MC1061	
JRR27	PY79	(1)
JRR48	Δ*rnhC*	(2)
JRR25	Δ*rnhB*	(2)
JRR33	Δ*rnhC rnhB::erm*	This work
JWS235	Δ*polA*	This work
JRR64	Δ*rnhC* Δ*polA*	This work
JRR94	Δ*ypcP*	This work
JRR96	Δ*rnhC* Δ*ypcP*	This work
JWS236	Δ*rnhB* Δ*polA*	This work

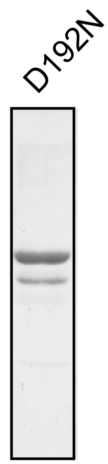
Table S3. List of primers

Name	Sequence
oJR338	/5IRD700/GCATCAAGCGCG
oJR339	rArGrUrArGrUrGrArArCrCrATGCTTACG/3IR800CWN/
oJR340	CGTAAGCATGGTTCACTACTCGCGCTTGATGC
oJR343	CGTAAGCATGGTTCACTACTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTT
oJR348	AGTAGTGAACCATGCTTACG/3IR800CWN/
oJR361	GCAATCGACTCGTAAGCATGGTTCACTACTCGCTGCTTGATGCTCAATCG
oJR362	/5IRD800/CGATTGAGCATCAAGCAGCG
oJR363	/5IRD800/CGATTGAGCATCAAGCAGCGAGTAGTGAACCATGCTTACGAGTCGATTGC
oJR364	/5IRD800/CGATTGAGCATCAAGCAGCGAGTAGTGAACCA
oJR365	CGTAAGCATGGTTCACTACT
oJR366	GCATCAAGCGCGAGATCACT
oJR367	rArGrUrArGrUrGrArArCrCrATGCTTACGAGTCGATTGC/3IRD700/
oJR368	CGTAAGCATGGTAGTGATCTCGCGCTTGATGC
oJR102	CCGCGAACAGATTGGAGGTATGACGGAACGAAAAAAATTAGTGC
oJR103	TGGTGGTGGTGGTGCTCGATTATTTCGCATCGTACCAAGATGG
oJR153	CGCGAACAGATTGGAGGTATGAATAATAATAAACTATTGCTGGTTGACGG
oJR154	GTGGTGGTGGTGGTGCTCGACTAAACGATCTCTCTAGCGTTCAGC
oJR378	GCAGCGGCTGCCTGTATCGGTACTCTTGCCG
oJR379	CAGGCAGCCGCTGCATAGCCGGCAAAGCC

Red text represents RNA, IRD indicates infrared dye, 700 or 800 indicates excitation at 700 nM or 800 nM, respectively, CWN is NHS ester conjugation, 5 or 3 indicates 5' or 3' end label.

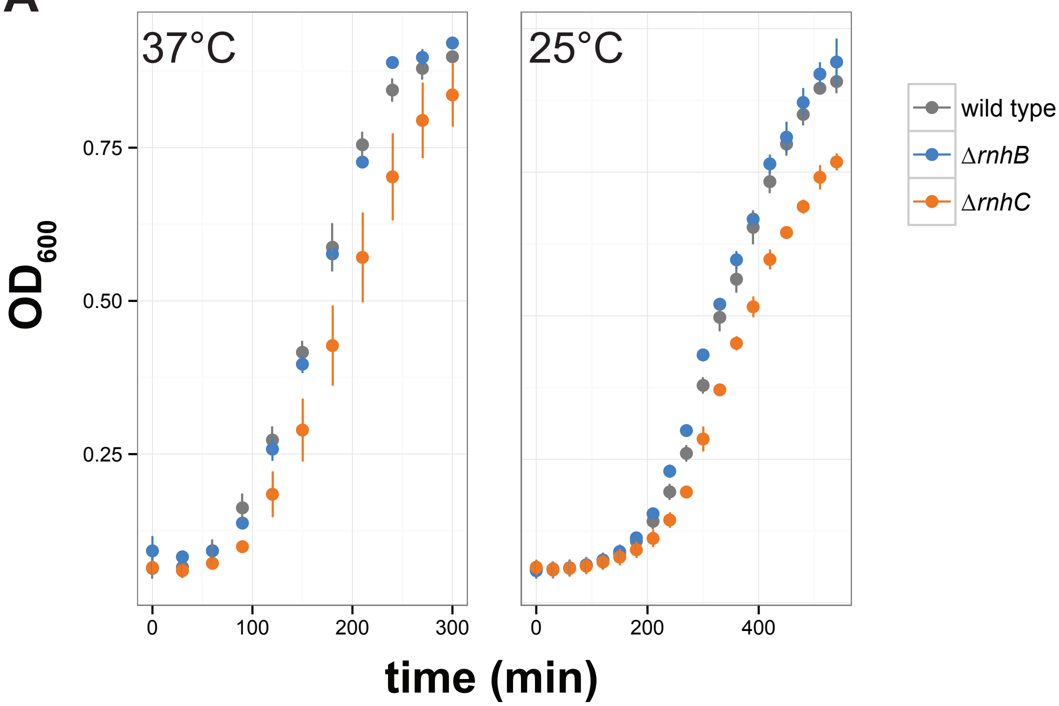
Table S4. List of plasmids

Plasmid	Vector	Insert
pDR244	N/A	N/A (BGSC)
pJR22	pE-SUMO	*polA* (3)
pJR31	pE-SUMO	*ypcP*
pJR17	pE-SUMO	*rnhB* (3)
pJR19	pE-SUMO	*rnhC* (3)
pJR82	pE-SUMO	*ypcP D192N*



Supplemental Figure S1. SDS-PAGE of purified YpcP D192N stained with Coomassie Brilliant Blue.

A



B

	Strain	Growth rate (μ_m)	95% CI	Doubling time
37°C	PY79	5.05	(4.41,5.68)	137
	Δ*rnhB*	5.14	(4.32,5.94)	135
	Δ*rnhC*	4.19	(3.49,4.88)	165
25°C	PY79	2.86	(2.65,3.07)	242
	Δ*rnhB*	2.85	(2.66,3.03)	243
	Δ*rnhC*	2.24	(2.11,2.38)	309

Growth rate estimates are multipled by 1,000.

Growth rate estimate is the maximum OD_{600}/time over the linear portion of the model.

Doubling time estimates are given in minutes.

Supplemental Figure S2. Cells with Δ*rnhC* have a slow growth phenotype at 25°C. **(A)** Growth curves for wild-type and RNase H deficient strains at 37°C and 25°C. Three biological replicates were averaged at each time point and the standard error is represented by vertical bars. **(B)** Growth curves were fit to a modified Gompertz growth model (4). The estimated growth rate and 95% confidence intervals from the fit are indicated. Estimated doubling times were calculated as ln(2)/μ_m where μ_m is the estimated growth rate obtained from the modified Gompertz growth model.

REFERENCES

1. Youngman P, Perkins JB, Losick R. 1984. Construction of a cloning site near one end of Tn917 into which foreign DNA may be inserted without affecting transposition in Bacillus subtilis or expression of the transposon-borne erm gene. Plasmid 12:1-9.

2. Yao NY, Schroeder JW, Yurieva O, Simmons LA, O'Donnell ME. 2013. Cost of rNTP/dNTP pool imbalance at the replication fork. Proceedings of the National Academy of Sciences of the United States of America 110:12942-7.

3. Randall JR, Hirst WG, Simmons LA. 2017. Substrate specificity for bacterial RNase HII and HIII is influenced by metal availability. J Bacteriol doi:10.1128/JB.00401-17.

4. Zwietering MH, Jongenburger I, Rombouts FM, van 't Riet K. 1990. Modeling of the bacterial growth curve. Appl Environ Microbiol 56:1875-81.