RNase E-dependent degradation of *tnaA* mRNA encoding tryptophanase is prerequisite for the induction of acid resistance in *Escherichia coli*

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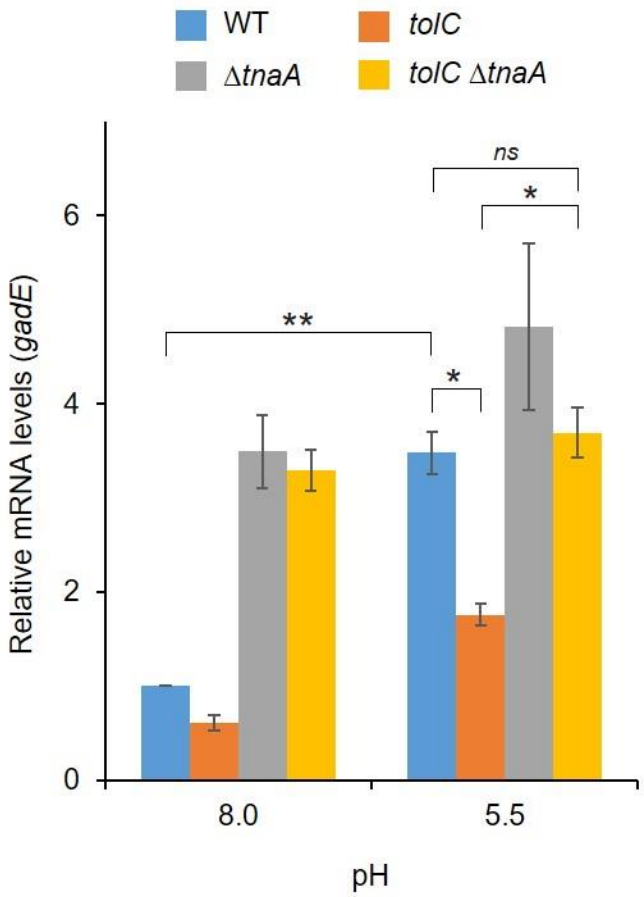
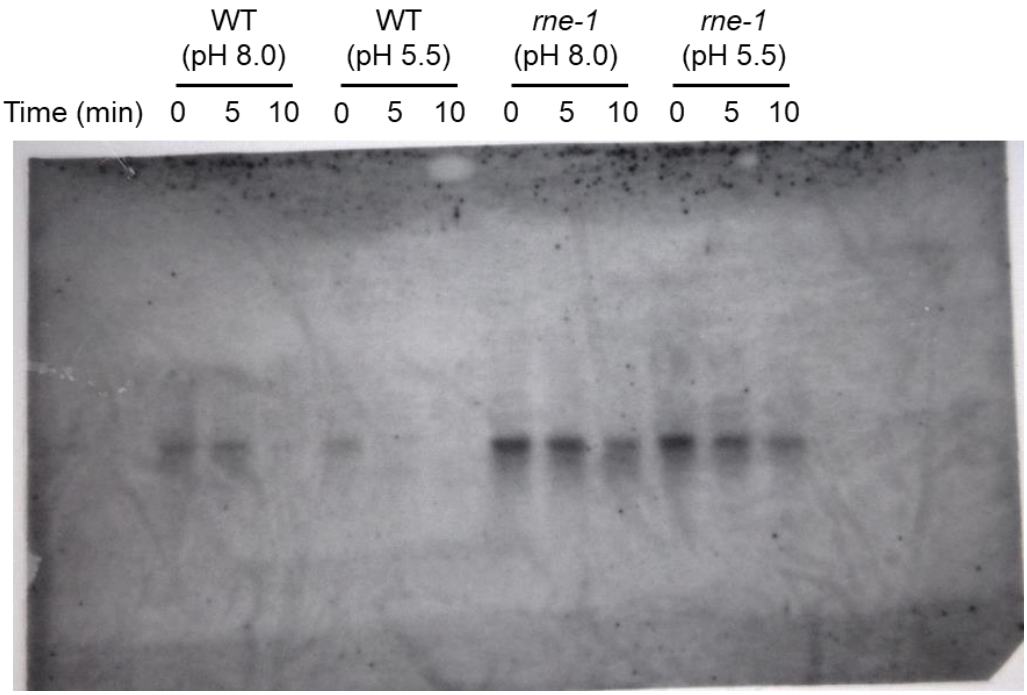
Supplementary Figure S1: Relative mRNA levels of *gadE* in MG1655 and its derivative mutants at pH 8.0 or 5.5



Relative mRNA levels of *gadE* in *E. coli* MG1655 (WT) and its derivative mutants grown at pH 8.0 or 5.5 were quantified using qRT-PCR. The *gadE*-mRNA levels were normalised to the reference gene transcript (16S rRNA) from the same RNA samples. Values are presented as means ± SEM from three independent experiments ($n = 3$) and were analysed using one-way ANOVA with Bonferroni post-hoc test (*$p < 0.05$, **$p < 0.01$).

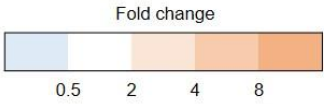
Supplementary Figure S2: A whole image of the northern blot of Fig. 3b.



The photo was taken by a CCD camera and the image was not processed by any image processing softwares.

Supplementary Table S1: RPKM ratios of genes encoding core enzymes and regulators of the GAD system in MG1655 and its *tolC* mutant cells at pH 8.0 or 5.5.

Gene	Product	pH 5.5/pH 8.0		*tolC*/WT	
		WT	*tolC*	pH 8.0	pH 5.5
gadW	Transcriptional activator of *gadA* and *gadBC*, and repressor of *gadX*	11.76	8.70	0.75	0.55
gadA	Glutamate decarboxylase A	9.36	9.89	0.49	0.51
gadE	GAD regulon activator	7.67	5.13	0.72	0.48
gadX	Acid resistance regulon transcriptional activator	6.75	2.89	0.97	0.41
gadY	Antisense sRNA, which positively regulates *gadX* and *gadW*	6.67	3.14	1.01	0.47
dsrA	Antisense sRNA, which enhances translation of *rpoS*	6.55	3.53	1.96	1.05
arrS	Antisense sRNA, which enhances transcription of *gadE*	6.02	4.16	0.68	0.47
gadC	Glutamate:gamma-aminobutyric acid antiporter	2.27	2.75	0.58	0.70
gadB	Glutamate decarboxylase B	2.13	2.76	0.56	0.73
torR	Response regulator in two-component regulatory system with TorS	2.07	2.30	0.93	1.03
phoP	Response regulator in two-component regulatory system with PhoQ	2.02	1.89	1.02	0.95
phoQ	Sensory histidine kinase in two-component regulatory system with PhoP	1.79	1.56	1.03	0.90
ydeO	UV-inducible global regulator	1.78	0.96	0.86	0.46
evgS	Hybrid sensory histidine kinase in two-component regulatory system with EvgA	1.63	0.91	1.09	0.61
crp	cAMP-activated global transcription factor, which mediates catabolite repression	1.63	1.62	0.69	0.68
evgA	Response regulator in two-component regulatory system with EvgS	1.36	0.76	0.87	0.48
torS	Hybrid sensory histidine kinase in two-component regulatory system with TorR	1.35	1.02	0.96	0.72
hns	Global DNA-binding transcriptional dual regulator	1.09	0.95	1.04	0.91
lon	DNA-binding ATP-dependent protease	1.04	1.05	0.77	0.78
sspA	Stringent starvation protein A	1.03	1.08	0.95	0.98
mnmE	tRNA U34 5-methylaminomethyl-2-thiouridine modification GTPase	1.02	0.93	1.01	0.92
rpoD	Sigma D (sigma 70) factor	1.01	1.50	0.95	1.42
gcvB	Antisense sRNA, which represses *oppA*, *dppA*, *gltI* and *livJ*	0.99	0.48	1.36	0.65
rpoS	Sigma S (sigma 38) factor	0.90	1.15	0.95	1.20
topA	DNA topoisomerase I	0.74	0.68	0.98	0.90
rne	Endoribonuclease	0.72	0.84	0.83	0.97



Supplementary Table S2: RPKM ratios for representative RNA substrates of RNase E upon acid treatment in MG1655 cells.

Gene	Product	pH 5.5/pH 8.0 (WT)	Reference
dnaG	DNA primase	0.86	1
ompA	Outer membrane protein A	1.14	2
ptsG	Glucose-specific PTS enzyme, IIB component	1.63	3
rne	Endoribonuclease E	0.72	4
rpsO	30S ribosomal subunit protein S15	0.93	5
rpsT	30S ribosomal subunit protein S20	0.84	6
ryhB	sRNA antisense regulator mediating positive Fur regulon response	1.29	2

PTS, phosphoenolpyruvate: carbohydrate phosphotransferase system

Supplementary Table S3: RPKM ratios of genes encoding *tna*-operon regulators upon acid treatment in MG1655 and its *tolC* mutant cells.

Gene	Product	pH 5.5/pH 8.0		Reference
		WT	*tolC*	
torS	Hybrid sensory histidine kinase in two-component regulatory system with TorR	1.35	1.02	7
torR	Response regulator in two-component regulatory system with TorS	2.07	2.30	7
torI	Response regulator inhibitor for *tor* operon	1.07	0.45	8
crp	cAMP-activated global transcription factor, mediator of catabolite repression	1.63	1.62	9
cyaA	Adenylate cyclase	1.20	1.51	10

Supplementary Table S4: Bacterial strains used in this study.

Strain	Relevant genetic marker(s)	Donor (Reference)	Source or reference
MG1655	Wild type		Laboratory stock
MG1655T	MG1655 *tolC*::Tn*10*		11
TK20	MG1655 Δ*tnaA*::*kan*	JW3686 (Keio collection[12])	This study
TK12	MG1655T Δ*tnaA*::*kan*	JW3686 (Keio collection[12])	This study
TK30	MG1655 *rng*::*cat*	GW11[13]	This study
TK40	MG1655 *rne-1*	GW20[13]	This study
TK34	TK30 *rne-1*	GW20[13]	This study
TK50	MG1655 *hfq10*::*cat*	HAT10[14]	This study
TK60	MG1655 *rne-105* (*smb-105*)	BZ5[15]	This study

Mutant strains were constructed by P1 phage-mediated transduction using MG1655 or its derivatives as acceptors and strains carrying the appropriate mutations as donors.

Supplementary Table S5: DNA primers used for qRT-PCR analysis.

Target gene	5′ primer sequence (5′→3′)	3′ primer sequence (5′→3′)	Reference
16S rRNA	GTTAATACCTTTGCTCATTGA	ACCAGGGTATCTAATCCTGTT	16
gadA	TTACCAGGTTGCCGCTTATC	ACGCAGACGTTCAGAGAGGT	17
tnaA	CTTTAAACATCTCCCTGAACCGTTC	GTGCCGCTGTCGGTCAGTAAATCG	This study
gadE	CTTTTCTTTTACAGGGCTTTTGGCAG	CGCTTCTTCATCAAGGATATGATTG	This study
rne	GAATGTTAATCAACGCAACTCAGC	GGTTCAATGCGGGTGATTTTAC	This study
rng	CTGAATTGTTAGTAAACGTAACGCC	AAGTACACGACTTACACGACCCTTG	This study

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