Supplementary Figures and Tables

The RNA ligase RtcB reverses

MazF-induced ribosome heterogeneity in *Escherichia coli*

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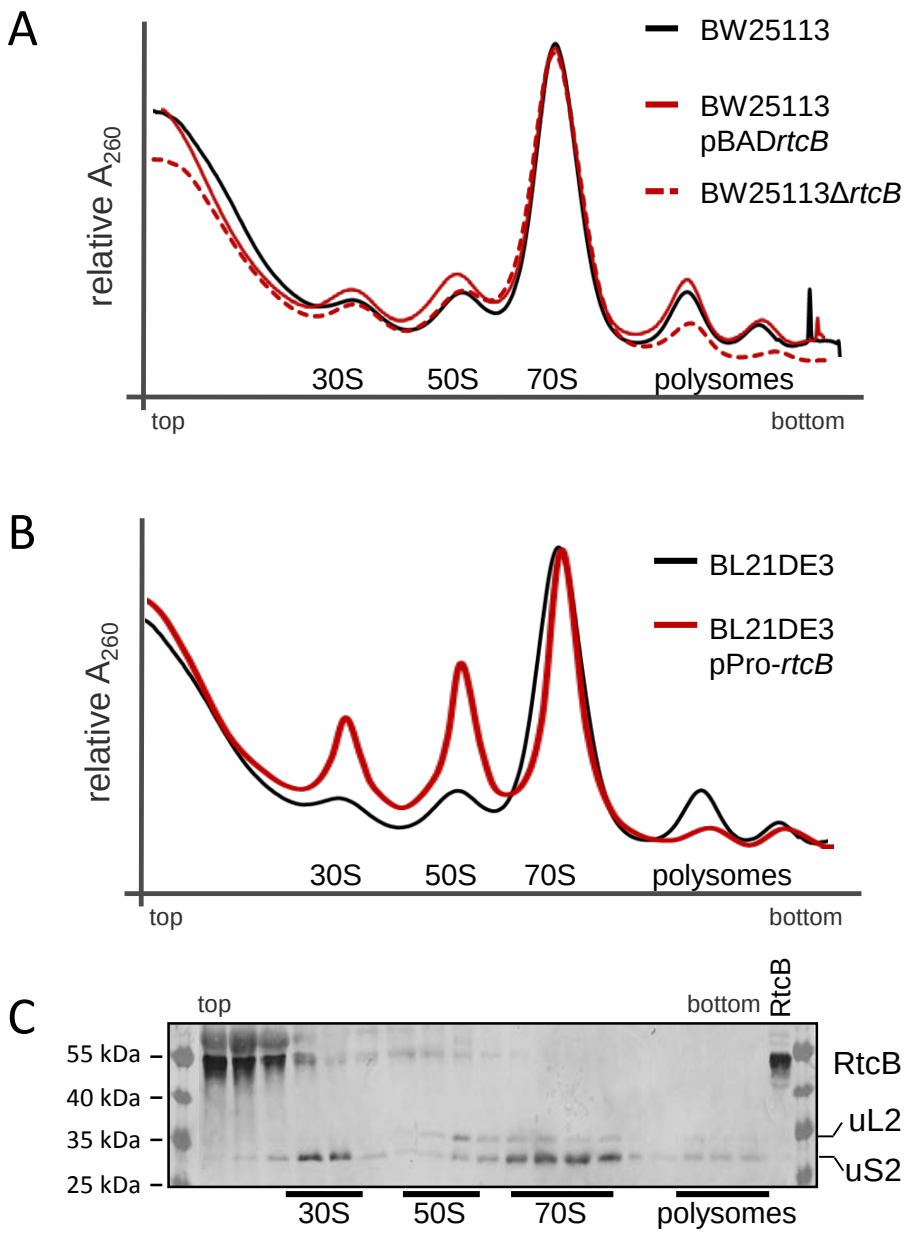
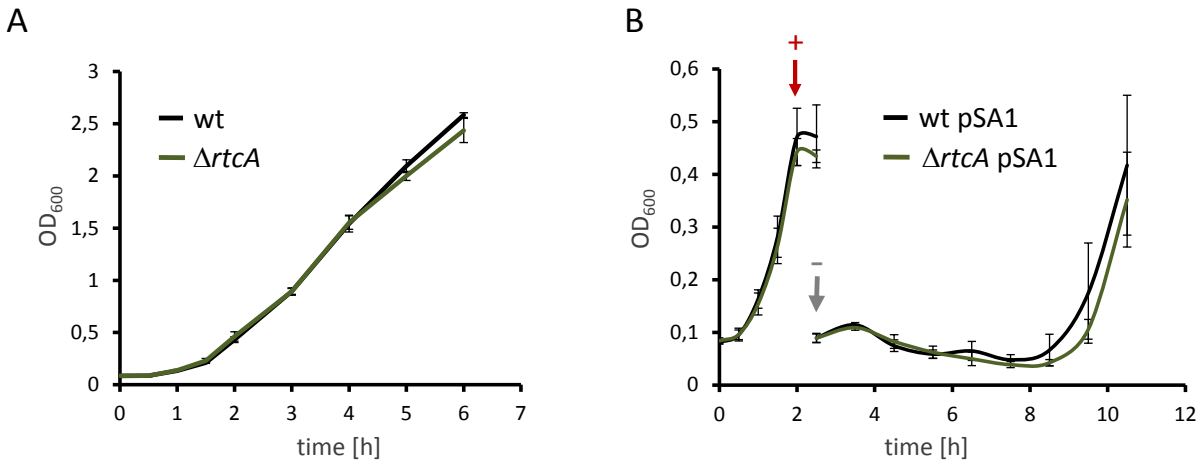
SUPPLEMENTARY FIGURES



Supplementary Figure S1. RtcB affects the SU/M ratio but does not alter the sedimentation of 70S ribosomes or ribosomal subunits. For ribosome profile analysis S30 extracts were prepared from exponentially growing *E. coli* strains BW25113, BW25113Δ*rtcB* and from strain BW25113 harboring plasmid pBAD*rtcB* 30 minutes after induction of *rtcB* expression by addition of 0.2% arabinose (**A**) or from strain BL21DE3 before and 30 minutes after induction of *rtcB* expression from plasmid pET*rtcB* by addition of 0.1mM IPTG (**B**). The extracts were separated on 10-30% sucrose gradients in Tico buffer (20 mM Hepes pH 7.4 at 4°C; 6 mM MgOAc; 30 mM NH_4Ac; 4 mM 2-mercapthoethanol) and analyzed and

fractionated using an Äkta FPLC system (GE Healthcare, NJ, USA). **C** The proteins from the respective fractions were precipitated using TCA and further tested for the presence of RtcB using an RtcB specific antibody. Purified RtcB was used as control. Antibodies specific for ribosomal proteins uS2 and uL2 were used to indicate the fractions comprising the subunits, 70S monosomes and polysomes, respectively. The molecular weights of the marker proteins are given to the left.



Supplementary Figure S2. RtcA does not affect growth and recovery after *mazF* expression. **A** Growth of strain MC4100 F´ (black) and its isogenic *rtcA* deletion mutant (green) was monitored by measuring the OD_{600}. See also Figure 4A. **B** Strain MC4100 F´ (black) and its isogenic *rtcA* deletion mutant (green) both harboring plasmid pSA1 were grown to an OD_{600} of 0.35, when *mazF* expression was induced by addition of 1 mM IPTG (+). 30 minutes thereafter cells were transferred in fresh medium to remove the inductive agent (-) and growth recovery was monitored for additional 7 hours. The analysis was performed in triplicate, error bars indicate the standard deviation from the mean. See also Figure 4B.

SUPPLEMENTARY TABLES

Supplementary Table 1

Gene	Protein product	Log_2 fold change
rplY	50S ribosomal protein L25	1,1614
rpsP	30S ribosomal protein S16	1,1461
rpsF	30S ribosomal protein S6	1,1139
rplJ	50S ribosomal protein L10	1,1139
rpsM	30S ribosomal protein S13	1,0000
rplS	50S ribosomal protein L19	1,0000
rplX	50S ribosomal protein L24	0,9700
rplC	50S ribosomal protein L3	0,9542
rplU	50S ribosomal protein L21	0,9542
rpsA	30S ribosomal protein S1	0,9420
rpsD	30S ribosomal protein S4	0,9294
rpmG	50S ribosomal protein L33	0,9031
rpsU	30S ribosomal protein S21	0,9031
rplW	50S ribosomal protein L23	0,9031
rpmF	50S ribosomal protein L32	0,8451
rplI	50S ribosomal protein L9	0,8239
rpSJ	30S ribosomal protein S10	0,8129
rpsE	30S ribosomal protein S5	0,7782
rpsG	30S ribosomal protein S7	0,7782
rpmA	50S ribosomal protein L27	0,7782
secB	protein export chaperone, general protein chaperone	0,7782
rplA	50S ribosomal protein L1	0,7533
rplF	50S ribosomal protein L6	0,7270
rplO	50S ribosomal protein L15	0,6990
rplR	50S ribosomal protein L18	0,6990
trmD	tRNA (guanine-N(1)-)-methyltransferase	0,6990
gpsA	glycerol-3-phosphate dehydrogenase [NAD(P)+]	0,6990
pheA	P-protein chorismate mutase	0,6990
ydcY	DUF2526 family protein	0,6990
rplM	50S ribosomal protein L13	0,6601
lon	Lon protease	0,6532
ycbX	6-N-hydroxylaminopurine detoxification oxidoreductase	0,6021
rplL	50S ribosomal protein L7/L12	0,5836
yahK	aldehyde reductase, NADPH-dependent, Zn-containing	0,5441
rplQ	50S ribosomal protein L17	0,5119
rpsQ	30S ribosomal protein S17	0,4771
ribB	3,4-dihydroxy-2-butanone 4-phosphate synthase	0,4771
nanK	N-acetylmannosamine kinase	0,4771
ynfG	S- and N-oxide reductase, Fe-S binding	0,4771
cspD	cold shock-like protein CspD	0,4771
hns	DNA-binding protein H-NS	0,4771
yfeX	porphyrinogen oxidase, cytoplasmic	0,4771
rpsP	30S ribosomal protein S15	0,3979

rpsR	30S ribosomal protein S18	0,3979
rplT	50S ribosomal protein L20	0,3979
rplE	50S ribosomal protein L5	0,3979
nfuA	Fe/S biogenesis protein NfuA	0,3979
luxS	S-ribosylhomocysteine lyase	0,3680
grcA	autonomous glycyl radical cofactor A	0,3522
ydgH	DUF1471 family periplasmic tri-domain protein	0,3522
uspG	universal stress protein G	0,3522
rsuA	ribosomal small subunit pseudouridine synthase A	0,3010
groL	60 kDa chaperonin GroEL	0,3010
tig	trigger factor	0,3010
groS	10 kDa chaperonin GroES	0,3010
mglB	D-galactose-, D-glucose-binding protein, periplasmic	0,3010
infC	translation initiation factor IF-3	0,3010
xseB	exonuclease VII, small subunit	0,3010
mdh	malate dehydrogenase	0,3010
bfr	bacterioferritin	0,3010
yacF	FtsZ stabilizer	0,3010

Supplementary Table 1. Proteins that interact with RtcB as identified by co-purification and subsequent mass-spectrometry. Proteins enriched by ratios > 0,3 log2 fold-change in relation to the mock experiment were considered significant.

Supplementary Table 2

	Relevant features	Source or reference
***E. coli* strains**		
MG1655	F^-, lambda$^-$, *rph*-1	(1)
JE28	MG1655::*rplL-his*	(2)
BW25113	F^-, DE(*araD-araB*)567, *lacZ4787*(del)::*rrnB*-3, LAM-, *rph*-1, DE(*rhaD-rhaB*)568, *hsdR*514	(3)
MC4100 F´	[*araD*139]B/r, Del(*argF-lac*)169, lambda$^-$, e14-, *flhD*5301, Δ(*fruK-yeiR*)725(*fruA*25), *relA*1, *rpsL*150(str^R), *rbsR*22, Del(*fimB-fimE*)632(::IS1), *deoC*1, [F' *proAB* $lacI^q$ZΔM15, Tn10 (Tet^R)]	(4)
BL21(DE3)	*dcm*, *ompT*, *hsdS*(rB^-mB^-), *gal*, λDE3	NEB
MG1655Δ*rtcB*	MG1655, $rtcB^-$	this study
BW25113Δ*rtcB*	BW25113, $rtcB^-$	this study
MC4100Δ*rtcB* F´	MC4100 F', $rtcB^-$	this study
Plasmids		
pSA1	amp^R, pQE30 derivative harboring *mazF* gene	(5)
pBAD-*rtcB*	cam^R, pBAD33 derivative harboring *mazF* gene	this study
pProEX-HTb	amp^R, vector for Trc driven gene expression	Invitrogen
pPro-*gfp*	amp^R, pProEX-HTb encoding his- and HA-tagged emGFP	this study
pPro-*rtcB*	amp^R, pProEX-HTb encoding his- and HA-tagged RtcB	this study
pPro-*rtcB*(-His)	amp^R, pProEX-HTb encoding HA-tagged RtcB	this study
pET28a	kan^R, vector for T7 driven gene expression	Novagen
pET*rtcB*	kan^R, pET28a derivative harboring *rtcB* gene	this study
pTwin1	amp^R, vector for protein purification *via* an intein tag	NEB
pTwin*rtcB*	amp^R, pTwin1 derivative harboring *rtcB* gene	this study
706-Flp	tet^R, encoding Flp recombinase	Gene Bridges
pACA-RNA43^{SD}	cam^R, pBAD33 derivative harboring the 3´-terminal 54 nts of *rrnB* followed by *glyT* and the ara terminator	this study
pGCA-RNA43^{SD}	pACA-RNA43^{SD}, ACA at position 1500 of the *rrnB* fragment was changed to GCA	this study
pUH-C_ΔACA-EmGFP	amp^R, pUH21-2 derivative harboring the emerald-gfp gene devoid of ACA sites under control of the PA1-04/03 promoter	Oron-Gottesman et al., under revision
p*gfp*^{aSD}	pUH-C_ΔACA-EmGFP derivative displaying an aSD sequence upstream of the *gfp* start codon	this study

Supplementary Table S2. Bacterial strains and plasmids used in this study

Supplementary Table 3

Name	Binding region	Sequence*
PCR amplification of DNA templates for cloning		
$D10_{fwd}$	*rtcB* from nt 1 - 17	ATAT*CCATGG*GTATGAATTACGAATTACT
$rtcB_{rev}$	*rtcB* from nt 1227 – 1209	AAGGATCCTCATCATTATCCTTTTACGCACACC
IM_$P9_{fw}$	*rtcB* from nt 1 – 18	TA*CCATG*GTATACCCATACGATGTTCCAGATTACGCTAT GAATTACGAATTACTG
IM_$R9_{rev}$	*rtcB* from nt 1227 – 1209	AA*CTCGAG*TCATCATTATCCTTTTACGCACACC
IM_$I19_{rev}$	pUH-C_ΔACA-EmGFP (changing the SD to aSD)	[phos] GGA GGATAG GATCCA AAATAC GCCATG
IM_$J19_{fw}$	pUH-C_ΔACA-EmGFP (changing the SD to aSD)	[phos] CGCAAAAAATGGTGAGCAAGG
PCR amplification of DNA templates for *in vitro* transcription		
$W11_{fwd}$	*rrsB* from nt 1491 - 1506	AAAT*CTAGA*GTAATACGACTCACTATAGGGAAGTCGTAACAAGGT
$O18_{rev}$	*rrsB* from nt 1542 - 1524	TAAGGAGGTGATCCAACCG
Probes for northern blot analyses		
A20	*rrsB* from nt 1541 - 1511	AAGGAGGTGATCCAACCGCAGGTTCCCCTACGGTTACC
R25	*rrfB* from nt 120 - 101	ATGCCTGGCAGTTCCCTACT
CD	*rrsB* from nt 955 - 939	CCACATGCTCCACCGC
Primer extension analysis		
IM_$Y25_{rev}$	*rtcB* from nt 124 -139	GCATTACCGCAATATG
RT-PCR primer		
$S7_{fwd}$	*rrsB* from nt 1360 – 1379	AGAATGCCACGGTGAATACG
$X15_{rev}$	*rrsB* from nt 1499 - 1483	TACGACTTCACCCCAGT
$Y12_{rev}$	*rrsB* from nt 1542 - 1522	TAAGGAGGTGATCCAACCGC
$H17_{rev}$	$rrsB^{AgeI}$ from nt 1538 – 1523	GAGGTGATCCAACCGAT
$S19_{rev}$	$rrsB^{SD}$ from nt 1542 - 1522	TATCCTCCTGATCCAACCGC
Overlap-PCR primer		
IM_$U12_{fw}$	*rrsB* from nt 1488-1511 (introducing mutation A1500G)	GGTGAAGTCGTAGCAAGGTAACCG
IM_$V12_{rev}$	*rrsB* from nt 1488-1511 (introducing mutations A1500G)	CGGTTACCTTGCTACGACTTCACC
IM_$I15_{fwd}$	*rrsB* from nt 702 – 720	AG*AGATCT*GGAGGAATACC
IM_$J15_{fwd}$	*rrsB* from nt 1511 – 1530 (introducing mutations T1512G; A1513T; T1522A; G1523T)	GGTGGGGAACCATCGGTTGG
IM_$H15_{rev}$	pKK3535 from nt 3537 – 3552	CC*TCTAGA*CGAAGGGG
IM_$K15_{rev}$	*rrsB* from nt 1525 – 1504 (introducing mutations T1512G; A1513T; T1522A; G1523T)	CGATGGTTCCCCACCGGTTACC

* The T7 promoter sequence is underlined. Restriction sites are in italics.

Supplementary Table S3. Oligonucleotides used in this study

REFRENCES

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