SI Material and Methods

Monitoring of cell growth and viability

Bacterial strains used are listed in Table S1. *Staphylococcus* strains were grown in either complex medium TSB (tryptone soya broth, Oxoid) or in a chemically defined medium (CDM). The latter consisted of a basic salt medium (12.5 mM Na_2HPO_4, 10 mM KH_2PO_4, 1.65 mM $MgSO_4$, 9.25 mM NH_4Cl, 8.5 mM NaCl), 0.142 mM sodium citrate tribasic dihydrate, 75 mM α-D(+) glucose and 1 mM of all 19 L-amino acids. L-methionine (Met) was only added when indicated. Vitamins and trace elements were supplemented as follows (with final molarity in nM in brackets): cyanocobalamin (108), 4-aminobenzoic acid (870), D-biotin (120), nicotinamide (2430), Ca-D-panthothenic acid (630), pyridoxine hydrochloride (1860), thiamine hydrochloride (870), riboflavin (780), $ZnCl_2$ (510), $MnCl_2$ (500), H_3BO_3 (97), $CoCl_2$ (1460), $CuCl_2$ (15), $NiCl_2$ (100), Na_2MoO_4 (148) and $FeCl_3$ (750).
For strains carrying resistance genes, antibiotics were used at the following concentrations: 100 µg ml^{-1} for ampicillin, 10 µg ml^{-1} for erythromycin and 5 µg ml^{-1} for tetracycline. Overnight cultures were diluted in fresh medium to an optical density at 600 nm (OD_{600}) of 0.05 and a flask-to-medium ratio of 5:1. Cultures were grown at 37°C shaking (220 rpm). Initial growth was monitored as the OD_{600} of the culture over time, whereas long-term cell survival was measured by determining the CFU ml^{-1} at each time point indicated. For Figure 5 strains were first grown in CDM with Met. The cultures were filtered through a 0.22-µm filter with vacuum and washed twice with sterile phosphate-buffered saline (PBS). Bacteria were then resuspended in an equal volume of CDM with or without Met (with a flask-to-medium ratio of 2:1) and grown for another 60 min before sampled for RNA extraction.

Construction of strains

The conditional RNase J2 (*rnjB*) and RNase III (*rnc*) mutants were generated using the pMUTIN vector [1]. With this system, conditional mutants can be obtained by integrating the vector upstream of the target gene, which falls under the control of the isopropyl IPTG-inducible promoter P_{spac}. For this purpose a region encompassing the first 600/900 bp of the 5´ coding region of RNase J2 and RNase III was amplified from strain RN6390 by employing the primers listed in Table S5. The amplicons were then cloned into the *Eco*RI/*Bam*HI digested pMUTIN yielding vectors pCG106 and pCG107, respectively. The vectors were transferred into the restriction-deficient strain RN4220 under IPTG induction. pMUTIN insertion into the chromosome was verified by PCR and pulsed-field gel electrophoresis (PFGE). The mutations were then transduced into *S. aureus* strain Newman. Transductants were verified by PCR and PFGE.

RNA isolation and Northern blot analysis

Samples from bacterial cultures were mixed with 1X Vol. RNA protection solution (Qiagen) for immediate RNA stabilization. Cells were disrupted mechanically (Bertin technologies) and total RNA was purified using mini-scale, silica-membrane based spin-columns (Qiagen).

Northern blot analysis by agarose/ formaldehyde gels of two microgramm total RNA each sample followed standard procedures. Sequence-specific probes were generated by PCR with oligonucleotides listed in Table S3 (SI) and radioactively labeled with the DNA labeling system (Amersham, GE Healthcare) and [α^{32}P]-dCTP. For Figure 1B RNA probes were generated by T7 *in vitro* transcription with [α^{32}P]-CTP and the DNA probe was generated by 5' end-labeling with [γ^{32}P]-ATP and polynucleotide kinase. For clean-up from unincorporated nucleotides chromatography spin-columns with Bio-Gel P-6 (Bio Rad) were used. Signals were detected with the PhosphoImager (Fujifilm FLA-7000).

For Figure 5 RNA isolation and Northern blot analysis were performed as described [2]. Briefly, bacteria were lysed in Trizol reagent (Invitrogen) with zirconia-silica beads (0.1-mm diameter) in a high-speed homogenizer (Savant Instruments, Farming- dale, NY). RNA was isolated as described by the manufacturer of Trizol. Digoxigenin (DIG)-labeled probes were generated with a DIG-labeling PCR kit following the manufacturer's instructions (Roche Biochemicals) using oligonucleotides listed in Table S3.

Table S1 – Bacterial strains and plasmids used in this study.

Strain	Properties	Reference
Escherichia coli		
DH5α	common cloning host, *lacZ* negative	
Top10	Competent *E. coli* for plasmid transformation	Invitrogen
Staphylococcus aureus		
COL	methicillin resistant isolate (1960s)	[6]
N315	methicillin resistant isolate (1982)	[7]
Newman	methicillin-sensitive isolate (1952), NCTC 8178	[8,9]
Newman, 21	Newman *codY::tet*(M)	[10]
Newman, 86	Newman rsh_{syn} (nucleotides 942 to 950 deleted)	[11]
Newman, 106	Newman P_{spac}-*rnjB*, RNase J2 mutant	this work
Newman, 107	Newman P_{spac}-*rnc*, RNase III mutant	this work
RN4220	Restriction-deficient *S. aureus* strain, r-	[12]
RN6390	Laboratory strain derived from NCTC 8325	[13]
Staphylococcus epidermidis		
RP62A	biofilm-forming, *ica*-positive, IS*256*-positive, catheter sepsis isolate	ATCC 35984 [6]

Plasmids	Properties	Reference
pGEM-T Easy	linearised cloning vector system with 3' T overhang, Amp^r, *lacZ*	Promega
pGEM*met*COL	pGEM-T Easy with integration of 467 bp fragment of *met* leader RNA under T7 promoter control	this work
pMUTIN	Integrative vector including the IPTG-inducible promoter P_{spac}, Amp^r, Erm^r	[1]
pCG106	pMUTIN with integration of a 802 bp *rnjB* fragment for conditional mutagenesis	this work
pCG107	pMUTIN with integration of a 600 bp *rnc* fragment for conditional mutagenesis	this work

Table S2 – Overview of all mutations introduced in the *met* leader RNA template.

The numbers in the last column indicate the nucleotide position based on the *met* leader RNA sequence of *S. aureus* COL. The sequence of the oligonucleotides used for each construct are listed in Table S5.

Construct	Oligonucleotides	Site	Mutation
SC1	SB_ugc_1out Sa-SB_ugc_2out	**specifier box**	113AUG → 113**UGC**
SC2	Sa-TB_del_1out Sa-TB_del_2out	**T-box**	deletion of bases 360-366
SC3	Sa-TB_acca_1out Sa-TB_acca_2out	**T-box**	360UGGU → 360**ACCA**
SC4	Sa-TB_gg361cc_1out Sa-TB_gg361cc_2out	**T-box**	361GG → 361**CC**
SC5	Sa-TB_cc365gg_1out Sa-TB_cc365gg_2out	**T-box**	365CC → 365**GG**
SC6	Sa-TB_G361C_1out Sa-TB_G361C_2out	**T-box**	361G → 361**C**
SC7	Sa-TB_G362C_1out Sa-TB_G362C_2out	**T-box**	362G → 362**C**
SC8	Sa-TB_U363A_1out Sa-TB_U363A_2out	**T-box**	363U → 363**A**

Table S3 – Sequences of oligonucleotides used to generate hybridization probes.

The T7 RNA polymerase promoter sequence is underlined.

Gene	Primer	Sequence 5' → 3'	Probe
16S rDNA	forward	CCTTATGATTTGGGCTACACA	
	reverse	CCAGCTTCATATAGTCGAGTT	130 bp
***S. aureus* specific probes**			
met leader	forward	ATGTATTCTAAATGAGTCAGACAACC	
	reverse	CCGTCCTTCGTACCCGAATGA	588 bp
for sense RNA probe	T7-for	<u>TTTTCTAATACGACTCACTATAGGGAGAGGG</u>GAGGAAAGTAAAACACACCC	
	reverse	CCGTCCTTCGTACCCGAATGA	394 nt
for antisense RNA probe	T7-rev	<u>TTAACTAATACGACTCACTATAGGGAGAGGG</u>CCGTCCTTCGTACCCGAATGA	
	fow_nest	AGAGGAAAGTAAAACACACCCTG	394 nt
metI	forward	GCATCCAAAACTAGGACAATCGAC	
	reverse	CTCTCCAATCTGAGCTTTATCTAATGC	1006 bp
DIG-labeled probes (*S. aureus* specific)			
brnQ-1, permease	02923DIG-for	GTAAAGCCCAACCAACAGGT	
	02923DIG-rev	TCATCGTAGGTTTAACAGCA	321 bp
met leader	metRIBdigfor	CTTCAAGATACCAATTACAATTTC	
	metRIBdigrev	TTTGTTATTCCATCGCTGA	456 bp
metI	Nwmn_0351digfor	AAAATCCTACACGCTCAACA	
	Nwmn_0351digrev	GGTGTTGAAAGATAAGGTGTT	400 bp

Table S4 – Nucleotide sequences of RACE primers and oligonucleotides used in tRNA template PCR. T7 promoter sequence is underlined; additional nucleotides to the annotated tRNA sequence after the transcription reaction are highlighted in bold script.

RACE primer

Oligonucleotide	**Sequence 5' → 3'**
S. aureus specific	
sp1 = sp2	CGTGCGTAAAGAATCCAGTACGCC
sp3	AGACACCTCATATTGGCATCAAC
sp5	AGAGGAAAGTAAAACACACCCTG
nested sp5	AGTATGGGATAGCACATTACTATATCC
further nested sp5	ACTGAATAAGGTTATTTCAGCGATGG

primer for tRNA template

Oligonucleotide	**Sequence 5' → 3'**
Met12_tRNA_T7-F	CTAATACGACTCACTATA**G**CGCGGGATGGAGCAGTTCGGT
Met12_tRNA**cca**_R	**TGG**TTGCGGGAGGCGGATTTGAACCACC
Met12_tRNA**AdC**_R	**GTGG**TTGCGGGAGGCGGATTTGAACC
Cys_tRNA_T7-F	TTTTCTAATACGACTCACTATA**G**GGCGGCATAGCCAAGTGGT
Cys_tRNA**cca**_R	TGGAGGCGGCAACCGGATTTG
Cys_tRNA**AdC**_R	**G**TGGAGGCGGCAACCGGATTTG
Met3_tRNA_T7-F	TTAACTAATACGACTCACTATAGGACCTTTAGCTCAGTTGGT
Met3_tRNA**cca**_R	**TGG**TGGACCTTGCAGGACTCGA
Met3_tRNA_R	TGGACCTTGCAGGACTCGAACC
Met4_tRNA_T7-F	CTAATACGACTCACTATA**G**GGCGGTGTAGCTCAGCTGGC
Met4_tRNA**cca**_R	T**G**GTGGCGGTGGAGGGGATCGAACCC

Table S5 – Sequences of oligonucleotides used in SDM PCR and for generation of conditional RNase mutants. T7 promoter sequence and mutated nucleotides are underlined; position of deletion is indicated by an asterisk. The restriction sites *Eco*RI and *Bam*HI, respectively, are in italic.

SDM PCR primers

Oligonucleotide	**Sequence 5'→ 3'**
T7-F_met-sRNA	TTAACTAATACGACTCACTATAGGGAGATCTTATAACAGTTTAATGAAACGTAAAC
R_met-sRNA	GAAAAAATAAAAAAAGCTTCCGTCCTTCG
SB_ugc_1out	GAAATGCGCCTTGGAGTGTTGATGC
Sa-SB_ugc_2out	AGGCGCATTTCAAACACGCTTTCAA
Sa-TB_del_1out	AAAGG*GCGAAACATAAGCTTTCGTCC
Sa-TB_del_2out	TTCGC*CCTTTATTTGTTATTCCATCGC
Sa-TB_acca_1out	AAAGGACCAACCGCGAAACATAAGC
Sa-TB_acca_2out	TTCGCGGTTGGTCCTTTATTTGTTATTCCA
Sa-TB_cc365gg_1out	AAAGGTGGTAGGGCGAAACATAAGC
Sa-TB_cc365gg_2out	TTCGCCCTACCACCTTTATTTGTTATTCCATCG
Sa-TB_G362C_1out	AAAGGTGCTACCGCGAAACATAAGC
Sa-TB_G362C_2out	TTCGCGGTAGCACCTTTATTTGTTATTCCA
Sa-TB_G361C_1out	AAAGGTCGTACCGCGAAACATAAGC
Sa-TB_G361C_2out	TTCGCGGTACGACCTTTATTTGTTATTCCA
Sa-TB_gg361cc_1out	AAAGGTCCTACCGCGAAACATAAGC
Sa-TB_gg361cc_2out	TTCGCGGTAGGACCTTTATTTGTTATTCCA
Sa-TB_U363A_1out	AAAGGTGGAACCGCGAAACATAAGC
Sa-TB_U363A_2out	TTCGCGGTTCCACCTTTATTTGTTATTCCA

Conditional RNase mutants

RNase gene	**Oligonucleotide**	**Sequence 5' → 3'**
RNase J2	EcoSAV1275-for	GGGG*GAATTC*TAGGAGGTAAGATTTTGAG
	BamHSAV1275-rev	CCCC*GGATCC*TCAAGTGATCTTCCTAAA
RNase III	EcoSAV1233-for	GGGG*GAATTC*GCAACACATAAAGGAGAT
	BamHSAV1233-rev	AAAA*GGATCC*ATTATAGGTTACATCACC

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