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$_2$HPO$_4$, 10 mM KH$_2$PO$_4$, 1.65 mM MgSO$_4$, 9.25 mM NH$_4$Cl, 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$_3$BO$_3$ (97), CoCl$_2$ (1460), CuCl$_2$ (15), NiCl$_2$ (100), Na$_2$MoO$_4$ (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 ampiclons were then cloned into the EcoRI/BamHI 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 \(\alpha^{32}\text{P}\)-dCTP. For Figure 1B RNA probes were generated by T7 *in vitro* transcription with \(\alpha^{32}\text{P}\)-CTP and the DNA probe was generated by 5’ end-labeling with \(\gamma^{32}\text{P}\)-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 Phospholmager (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, Farmingdale, 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<sub>syn</sub> (nucleotides 942 to 950 deleted) | [11]       |
| Newman, 106       | Newman *P<sub>spac</sub>-rnjB*, RNase J2 mutant      | this work  |
| Newman, 107       | Newman *P<sub>spac</sub>-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, IS256-positive, catheter sepsis isolate | ATCC 35984 |

| Plasmids       | Properties                                           | Reference  |
|----------------|------------------------------------------------------|------------|
| pGEM-T Easy    | linearised cloning vector system with 3’ T overhang, *lacZ*, Amp<sup>r</sup> | Promega    |
| pGEM<sub>met</sub>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<sub>spac</sub>*, Amp<sup>r</sup>, Erm<sup>r</sup> | [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      | specifier box | 113AUG → 113UGC          |
| SC1       | Sa-SB_ugc_2out   |               |                           |
| SC2       | Sa-TB_del_1out   | T-box         | deletion of bases 360-366 |
| SC2       | Sa-TB_del_2out   |               |                           |
| SC3       | Sa-TB_acca_1out  | T-box         | 360UGGU → 360ACCA        |
| SC3       | Sa-TB_acca_2out  |               |                           |
| SC4       | Sa-TB_gg361cc_1out | T-box      | 361GG → 361CC           |
| SC4       | Sa-TB_gg361cc_2out|               |                           |
| SC5       | Sa-TB_cc365gg_1out | T-box     | 365CC → 365GG          |
| SC5       | Sa-TB_cc365gg_2out|               |                           |
| SC6       | Sa-TB_G361C_1out | T-box         | 361G → 361C            |
| SC6       | Sa-TB_G361C_2out |               |                           |
| SC7       | Sa-TB_G362C_1out | T-box         | 362G → 362C            |
| SC7       | Sa-TB_G362C_2out |               |                           |
| SC8       | Sa-TB_U363A_1out | T-box         | 363U → 363A            |
| SC8       | Sa-TB_U363A_2out |               |                           |
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                     | CCAGCTTCATATAGTGGAGTT              |       |
| 130 bp                      |        |                                   |       |
| **S. aureus specific probes** |        |                                   |       |
| *met* leader forward        | ATGTATTCTAAATGAGTCAGACAACC         | 588 bp|
| reverse                     | CCGTCCTTCGTACCCGAATGA              |       |
| for sense RNA probe T7-for | TTTTCTAATACGACTCTATAGGGAGAGGAAGATAAAACACACC | 394 nt|
| reverse                     | CCGTCCTTCGTACCCGAATGA              |       |
| for antisense RNA probe T7-rev | TTAACCTACGTCTACCTATAGGGAGAGGACCCTCTTCTGTACCGTCTCGTA | 394 nt|
| fow_nest                    | AGAGGAGATATAAACACACCCTG            |       |
| *metl*                      | GCATCCAAAACCTAGGACAATCGAC           | 1006 bp|
| reverse                     | CTCTCCATCTCGAGCTTTATCTATGC         |       |
| **DIG-labeled probes (S. aureus specific)** |        |                                   |       |
| *brnQ-1*, permease 02923DIG-for | GAAAGCCCAACACACAGGT | 321 bp|
| 02923DIG-rev                | TCATCGTAGGTTTAAACAGCA              |       |
| *met* leader 02923DIG-for   | CTTCAAGTACCAATTACATTTC             | 456 bp|
| metRIBdigestfor             | TTTGTTATTCCCATCGCTGA               |       |
| metRIBdigestrev             | AAAATCCTACAGCTCAACA                |       |
| Nwmn_0351digfor             | GGTGTTGAAAGATAAGGTGTT              | 400 bp|
| Nwmn_0351digrev             | GGTGTTGAAAGATAAGGTGTT              |       |
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 | CGTGCGTAAGAAATCCAGTACGCC |
| sp3 | AGACACCTCATATTGGGCATCAAC |
| sp5 | AGAGGAAGTAAAACACCCCTG |
| nested sp5 | AGTATGGGATAGCACATTATACCTATCC |
| further nested sp5 | ACTGAATAAGGTTATTTTCAGCGATGG |

| primer for tRNA template | Oligonucleotide | Sequence 5' → 3' |
|---------------------------|-----------------|-----------------|
| Met12_tRNA_T7-F | CTAATACGACTCATAATAGCGGGAATGGAGCAGTTGGT |
| Met12_tRNAcca_R | TGGTTGGGGAGGCGGATTTGACACC|
| Met12_tRNAAdC_R | GTGGTTGGGGAGGCGGATTTGAACC |
| Cys_tRNA_T7-F | TTTTCTAACGACTCATAATAGCGGGAATGGAGCAGTTGGT |
| Cys_tRNAcca_R | TGGAGGGGCAACCGGATTG |
| Cys_tRNAAdC_R | GTGGAGGGGCAACCGGATTG |
| Met3_tRNA_T7-F | TTAATACGACTCATAATAGCGGCACTTACCTATAGGAGCAGTTGGT |
| Met3_tRNAcca_R | TGGTTGACCTTGGCAGGACTCGA |
| Met3_tRNAAdC_R | TGGACCTTGGCAGGACTCGAACC |
| Met4_tRNA_T7-F | CTAATACGACTCATAATAGCGGGAATGGAGCAGCTCAGCTGGC |
| Met4_tRNAcca_R | TGGTTGGGGAGGCGGATCGACC |
| Met4_tRNAAdC_R | TGGTTGGGGAGGCGGATCGACC |
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 EcoRI and BamHI, respectively, are in italic.

**SDM PCR primers**

| Oligonucleotide | Sequence 5'→3' |
|-----------------|----------------|
| T7-F_met-sRNA   | TTAACTAATACGACTCACTATAGGGAGATCTTTATAACGATGAACGTAAAC |
| R_met-sRNA      | GAAAAAATAAAAAAGCTCCGTCTTTCG |
| SB_ugc_1out     | GAAAATGCGCCTTTGAGTTGATGC |
| Sa-SB_ugc_2out  | AGGCGCATTTTCACAACACGCTTTTCA |
| Sa-TB_del_1out  | AAAGG*GCGAACATAAGCTTTGCTCC |
| Sa-TB_del_2out  | TTCGC*CCTTTATTGTTATTCCATCGC |
| Sa-TB_acca_1out | AAAGGACCAACCGCAAACATAAGC |
| Sa-TB_acca_2out | TTCGCCGTTTGTCTTTATTTGTTATTCC |
| Sa-TB_cc365gg_1out | AAAGGTGTAAGGGCGAAACATAAGC |
| Sa-TB_cc365gg_2out | TTCGCCCTACCACTTTATTGTTATTTCCA |
| Sa-TB_G362C_1out | AAAGGTGCTACCGCAAACATAAGC |
| Sa-TB_G362C_2out | TTCGCCCTAGCACCTTTATTGTTATTTCCA |
| Sa-TB_G361C_1out | AAAGGTCTACCGCAAACATAAGC |
| Sa-TB_G361C_2out | TTCGCCGTACCCCTTTATTGTTATTTCCA |
| Sa-TB_gg361cc_1out | AAAGGTCTCTACCGCAAACATAAGC |
| Sa-TB_gg361cc_2out | TTCGCCGTTGACCTTTATTGTTATTTCCA |
| Sa-TB_U363A_1out | AAAGGTGGAACCGCAAACATAAGC |
| Sa-TB_U363A_2out | TTCGCCGTTTCCACCTTTATTTGTTATTTCCA |

**Conditional RNase mutants**

| RNase gene | Oligonucleotide | Sequence 5'→3' |
|------------|----------------|----------------|
| RNase J2   | EcoSAV1275-for | GGGGGATTCTAGGAGGTAAATTTGAG |
|            | BamHSAV1275-rev | CCCCCGATCTCAAGTGATCTTCTTCAA |
| RNase III  | EcoSAV1233-for | GGGGGATTTCGCAAACATAAAAGGAGAT |
|            | BamHSAV1233-rev | AAAAGGATCATTATAGGTTACATCACC |
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