SUPPLEMENTARY DATA

Construction of *B. subtilis rnpB* complementation plasmids

For complementation, the *B. subtilis rnpB* wild-type gene (*rnpB*wt) under control of its native *rnpB* promoter and terminator was cloned into pHY300 as described (1).

B. subtilis complementation plasmids under control of the xylose repressor (*xylR*) and promoter (P_{xyl}) were constructed in two steps: first, the fragment encoding *xylR* and P_{xyl} was amplified from plasmid pX2 (2) using phosphorylated primers "100", 5'-CGC GGA TCC AGG TTT GCT AAC CTT TGC G (introducing a BamHI site, underlined), and "105", 5'-GGG ACA CAT GTT ATC TCA TCA TAT ACA AAA TAA ATG TTT (introducing a PscI and a half SmaI site, underlined); after BamHI cleavage, the PCR product was cloned into pHY300 cut with SmaI and BamHI, resulting in plasmid pHY300 xylRP. *B. subtilis rnpB*wt was amplified from genomic DNA of strain W168 with primer "102", 5'-GAT GAG ATA ACA TGT TCT TAA CGT TCG GGT (introducing a region overlapping with the xylose promoter, underlined, at the 5'-end of *B. subtilis rnpB*), and primer "103", 5'-CGT CCC GGG CTT CAT CGT ATC ACC CTG TC (introducing a SmaI site 3'-adjacent to *rnpB*, underlined); the PCR product was cloned into the unique PscI and SmaI sites of pHY300 xylRP. This plasmid is referred to as pHY300 *B. subtilis* P_{xyl} *rnpB*wt (Table 1). Mutations C258 and C259 were introduced by the site-directed DpnI method according to the manual provided with the QuikChange XL Site-Directed mutagenesis Kit (Stratagene), using the primer pair 5'-TCC GTT AAG AAG GTT CCC CTA C**G**A AAA TTT GGG TTT CTC GCT CGA G and 5'- CTC GAG CGA GAA ACC CAA ATT TT**C** GTA GGG GAA CCT TCT TAA CGG A for the C258 mutation, and 5'-TCC GTT AAG AAG GTT CCC CTA **G**CA AAA TTT GGG TTT CTC GCT CGA G and 5'-CTC GAG CGA GAA ACC CAA ATT TTG **C**TA GGG GAA CCT TCT TAA CGG A for the C259 mutation (introduced point mutations underlined).

For parallel overexpression of the *B. subtilis* RNase P protein, *B. subtilis rnpA* including the xylose promoter was amplified from strain *sb* (3), using primers "115", 5'-CAG GAT CCG ATT TAG TAC ATA GCG AAT CTT ACC, and "116", 5'-CAG GAT CCA ACC AGA AAG GAA GCG C (both introducing a BamHI site, underlined); after BamHI cleavage, the PCR product was cloned into the BamHI site of pHY300 *B. subtilis* P_{xyl} *rnpB*(wt, 258 or 259). Constructs with the insert "P_{xyl} *rnpA*-stop" carried point mutations close to the 5'-end of the xylose-inducible *rnpA* gene, resulting in two stop codons; here, the xylose promoter region was amplified from genomic DNA of strain *sb* (3) with primer "114", 5'- TTT CGC TTC TTC AAA TGA CTC AT, and primer "115" (see above). The *rnpA* gene with the two stop codons was amplified from plasmid p3dstop (3) with primer "116" (see above) and primer "113", 5'- ATG AGT CAT TTG AAG AAG CGA AA. Those two fragments were combined by overlap extension PCR using primers "115" and "116", and the product was cloned into the BamHI site of pHY300 *B. subtilis* P_{xyl} *rnpB*(wt, 258 or 259).

Construction of *S. aureus rnpB* complementation plasmids

The *S. aureus rnpB* gene was cloned under control of the natural *B. subtilis rnpB* promoter and terminator. For amplification of the *B. subtilis* promoter, we used primer "19", 5'-GGC AGC AAG CTT TAT GAT TGA TCA C (including the naturally occurring HindIII site upstream of the *B. subtilis rnpB* promoter, underlined), and primer "44", 5'-ATG AAT TAT TAT ATA ACA ACT GAT TAC (covering the 3'-part of the *B. subtilis rnpB* promoter). The *B. subtilis* terminator was amplified using primer "45", 5'-ACA TTT AAA ATG ATG AAA ACA AGC, and primer "21", 5'-CGC CCA AGC TTG TGT ATA CTT CTT C (introducing a HindIII site, underlined, at the 3'-end of the *B. subtilis rnpB* terminator). *S. aureus rnpB* was amplified from genomic DNA of *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 12600) with primer "42", 5'-GTA ATC AGT TGT TAT ATA ATA ATT CAT TGA TAT

TTT GGG TAA TCG C, and primer "47", 5'-GCT TGT TTT CAT CAT TTT AAA TGT ACT AGT AGT GAT ATT TCT ATA AGC C (containing overlapping regions with *B. subtilis rnpB* promoter and terminator, respectively). Fragments encoding the *B. subtilis rnpB* promoter, the *S. aureus rnpB* coding region and the *B. subtilis rnpB* terminator were combined by overlap extension PCR. The resulting PCR product was cloned into the HindIII site of pHY300, yielding plasmid pHY300 *S. aureus* $P_{Bs\ rnpB}$ *rnpB*wt. Point mutations at position G238 and G239 within the *S. aureus rnpB* gene were introduced by the site-directed DpnI method as described above. For introduction of the C238 mutation, the following primers were used: 5'-CGA GTG AGC AAT CCA AAT TT**C** GTA GGA GCA CTT GTT TAA CGG and 5'-CCG TTA AAC AAG TGC TCC TAC **G**AA ATT TGG ATT GCT CAC TCG; for the C239 mutation: 5'-CGA GTG AGC AAT CCA AAT TT**G** CTA GGA GCA CTT GTT TAA CGG and 5'-CCG TTA AAC AAG TGC TCC TAG **C**AA ATT TGG ATT GCT CAC TCG (introduced point mutations are underlined). For the construction of pHY300 *S. aureus* $P_{Bs\ rnpB}$ *rnpB*(wt, 238 or 239) + P_{xyl} *rnp*A, the *B. subtilis rnpA* gene under control of the xylose promoter was amplified from strain *sb* (3) using phosphorylated primers "108", 5'-CCC GAT TTA GTA CAT AGC GAA TCT TAC C, and "107", 5'-GGG CAA CCA GAA AGG AAG CGC; the PCR product was cloned into the SmaI site of pHY300 *S. aureus* $P_{Bs\ rnpB}$ *rnpB*(wt, 238 or 239).

Construction of *E. coli rnpB* complementation plasmids

Complementation plasmids encoding *E. coli rnpB*wt, *rnpB*C292 and *rnpB*C293 under control of its native promoter ($P_{Ec\ rnpB}$) were cloned as described previously (1). *B. subtilis rnpA* under control of the xylose promoter was amplified from plasmid pHY300 *S. aureus* $P_{Bs\ rnpB}$ *rnpB*wt + P_{xyl} *rnp*A using primers 115 and 116 (see above, both introducing a BamHI site); PCR products were digested with BamHI and cloned into the BglII site of pHY300 *E. coli* $P_{Ec\ rnpB}$ *rnpB*(wt, 292 or 293).

Construction of pHY300 + P_{xyl} *rnpA*

B. subtilis rnpA under the control of the xylose-inducible promoter was amplified from plasmid pHY300 *B. subtilis* P_{xyl} *rnpB* + P_{xyl} *rnpA* with phosphorylated primers 107 and 108 (see above). The fragment was then cloned into the SmaI site of pHY300. The same was done for the xylose-inducible *rnpA* gene with two stop codons close to the 5'-end of *rnpA* (P_{xyl} *rnpA*-stop).

Construction of templates for T7 transcription

C258 and C259 mutations were introduced into *B. subtilis rnpB* by site-directed DpnI mutagenesis (see above) using pDW66 (4) as the template; pDW66 derivatives were linearized with DraI for T7 runoff transcription; *E. coli* P RNAs were transcribed from plasmid pJA2' linearized with FokI (5); $ptRNA^{Gly}U_{73}$, $ptRNA^{Gly}U_{73}$UAAAUA and $ptRNA^{Gly}U_{73}$CCAAUA were transcribed from PCR templates amplified with primer "167" (5'-ATT AAT ACG ACT CAC TAT AGG) as forward primer, and primer "169" (5'-AGC GGG AGA CGG GAC TTG), or primer "170" (5'- TAT TTA AGC GGG AGA CGG GAC TTG), or primer "171" (5'-TAT TGG AGC GGG AGA CGG GAC) as reverse primer, respectively; plasmid pSBpt3'HH (6) was used as the PCR template.

FIGURES

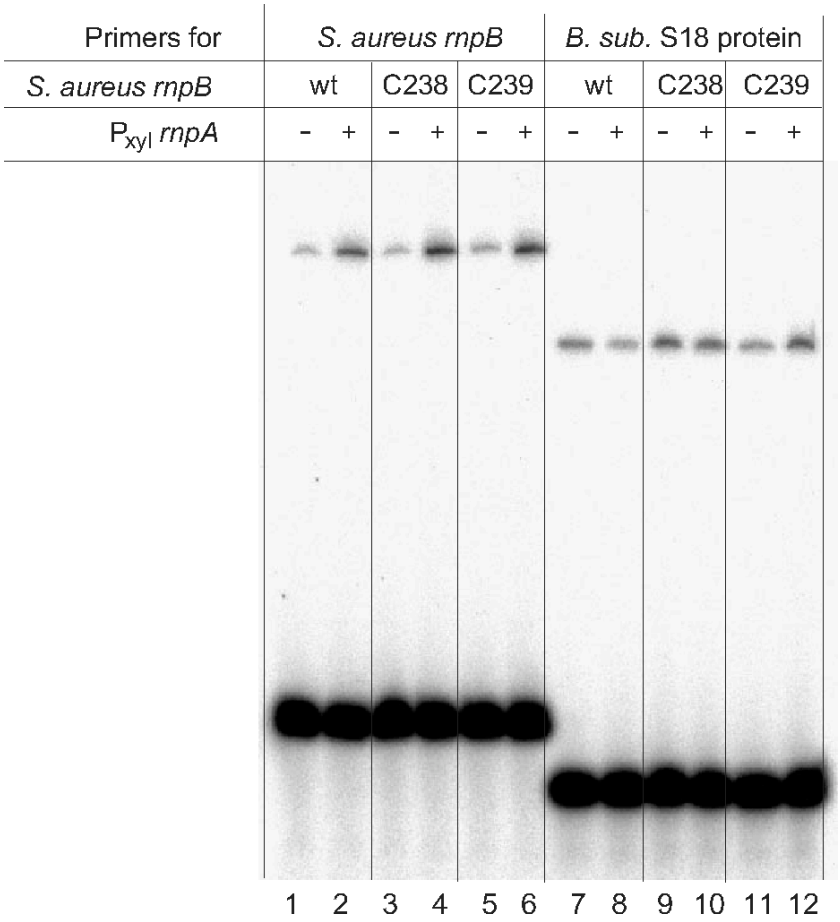

Figure S1. Radioactive reverse transcription PCR (RT-PCR) analysis of strain SSB318 complemented with *S. aureus rnpB*wt or *rnpB*C238/C239. PCR products were analyzed on a 10% polyacrylamide/8 M urea gel. Lanes 1–12: total RNA from SSB318 complemented with *S. aureus rnpB*wt (lanes 1, 2, 7, 8), *rnpB*C238 (lanes 3, 4, 9, 10) or *rnpB*C239 (lanes 5, 6, 11, 12) grown at 37°C in the absence of IPTG and in the presence of 2% xylose (w/v); 200 ng of total cellular RNA was used for RT-PCR. P_{xyl} *rnpA*: presence (+) or absence (–) of a xylose-inducible plasmid-encoded *B. subtilis rnpA* gene. Lanes 1-6: RT-PCR with primers (5'- CTA GTA GTG ATA TTT CTA TAA GCC ATG, 5'-endlabeled, and 5'- GGG TAA TCG CTA TAT TAT ATA GAG G) that covered the 5'- and 3'-terminal regions of *S. aureus* P RNA in order to detect full-length P RNAs; lanes 7-12: primers specific for the mRNA encoding *B. subtilis* ribosomal protein S18 (S18). For details on RT-PCR and primer sequences, see Material and Methods section of the main text.

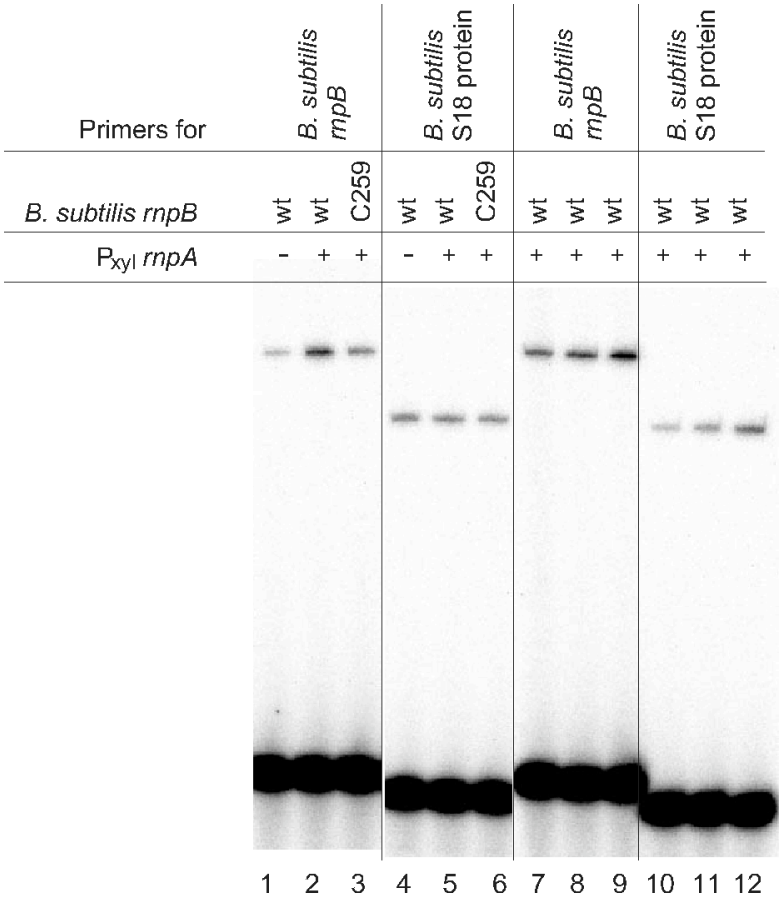

Figure S2. Radioactive reverse transcription PCR (RT-PCR) analysis of strain SSB318 complemented with *B. subtilis rnpB*wt or *rnpB*C259. PCR products were analyzed on a 10% polyacrylamide/8 M urea gel. Lanes 1–12: total RNA from SSB318 complemented with *B. subtilis rnpB*wt (wt, lanes 1, 2, 4, 5, 7-12) or *rnpB*C259 (C259, lanes 3 and 6) grown at 37°C in the absence of IPTG and in the presence of 2% xylose (w/v); amounts of total cellular RNA were 200 ng in lanes 1-6, 8 and 11, 100 ng in lanes 7 and 10, and 400 ng in lanes 9 and 12. P_{xyl} *rnpA*: presence (+) or absence (–) of a xylose-inducible, plasmid-encoded *B. subtilis rnpA* gene. Lanes 1-3 and 7-9: RT-PCR with primers (5'- AAG TGG TCT AAC GTT CTG TAA GCC, 5'-endlabeled, and 5'-CTT AAC GTT CGG GTA ATC GC) covering the 5'- and 3'-terminal regions of *B. subtilis* P RNA in order to detect full-length P RNAs; lanes 4-6 and 10-12: primers specific for the mRNA encoding *B. subtilis* ribosomal protein S18 (S18). For details on RT-PCR and primer sequences, see Material and Methods section of the main text. Lanes 7-12 document that the amount of RT-PCR product was sensitive to RNA template concentration.

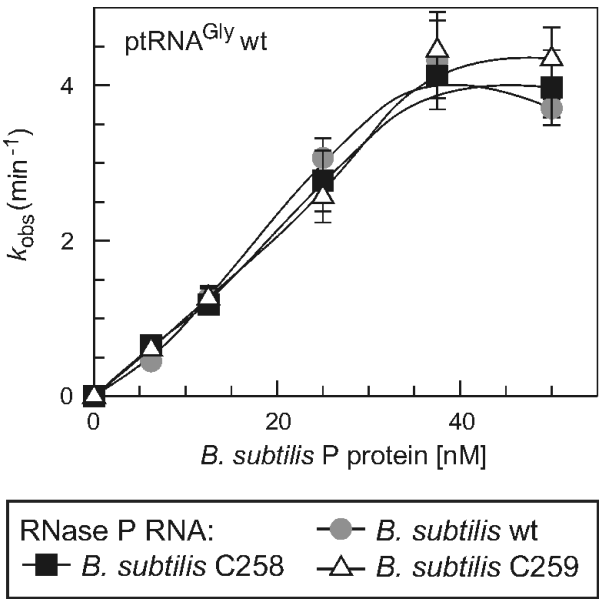

Figure S3. Activity of *in vitro* reconstituted *B. subtilis* RNase P wt and mutant holoenzymes as a function of protein concentration; k_{obs} is given as nmol substrate converted per nmol of P RNA per min; assay conditions: 50 mM Mes pH 6.1, 100 mM KCl and 10 mM $MgCl_2$; P RNA concentration was 20 nM, protein concentration as indicated and ptRNAwt concentration was 200 nM, including trace amounts (< 1 nM) of 5'-endlabeled substrate.

TABLES

Table S1. Heterologous complementation of *B. subtilis* RNase P mutant strain SSB318 by *E. coli* (type A) *rnp*B alleles

rnpB variants in pHY300	+ IPTG	- IPTG	aldose
E. coli $P_{Ec\ rnpB}$ *rnpB*wt	+ + +	+ + +	none
E. coli $P_{Ec\ rnpB}$ *rnpB*C292	+	-	none
E. coli $P_{Ec\ rnpB}$ *rnpB*C293	+	-	none
E. coli $P_{Ec\ rnpB}$ *rnpB*wt + P_{xyl} *rnp*A	n. d.		
E. coli $P_{Ec\ rnpB}$ *rnpB*C292 + P_{xyl} *rnp*A	+	-	xylose
E. coli $P_{Ec\ rnpB}$ *rnpB*C293 + P_{xyl} *rnp*A	+	-	xylose

Growth of mutant strain SSB318 transformed with wild-type *E. coli rnpB* (*rnpB*wt) and respective mutant alleles on plasmid pHY300; promoter types: $P_{Ec\ rnpB}$, native *E. coli rnpB* promoter; P_{xyl}, inducible xylose promoter; *B. subtilis rnpA* was overexpressed in parallel from the same plasmid as indicated. Cell growth was analyzed on LB plates in the presence (1 mM) or absence of IPTG; the aldose xylose was added to 2% (w/v) for pHY300 derivatives encoding *B. subtilis rnpA*.
+ + +: growth with equal numbers of colonies on +/- IPTG plates;
+: retarded cell growth;
-: no growth;
n. d.: not determined

Table S2. Cleavage rates at low Mg^{2+} concentrations for *B. subtilis* holoenzymes and *E. coli* hybrid holoenzymes reconstituted *in vitro*.

P RNA	ptRNA^{Gly}	enzyme concentration	[Mg^{2+}]	k_{obs}	k_{rel}
E. coli wt	wt	10 nM P RNA/37 nM RnpA	4.5	6.3 ± 0.02	1.0
E. coli C292	wt	10 nM P RNA/37 nM RnpA	4.5	2.3 ± 0.05	0.37
E. coli C293	wt	10 nM P RNA/37 nM RnpA	4.5	7.3 ± 0.08	1.16
E. coli wt	wt	10 nM P RNA/37 nM RnpA	2.0	7.3 ± 0.12	1.0
E. coli C292	wt	10 nM P RNA/37 nM RnpA	2.0	0.5 ± 0.01	0.07
E. coli C293	wt	10 nM P RNA/37 nM RnpA	2.0	1.9 ± 0.02	0.26
E. coli wt	wt	50 nM P RNA/185 nM RnpA	2.0	26.8 ± 0.41	1.0
E. coli C292	wt	50 nM P RNA/185 nM RnpA	2.0	1.5 ± 0.03	0.06
E. coli C293	wt	50 nM P RNA/185 nM RnpA	2.0	3.5 ± 0.02	0.13

Assay conditions: 20 mM Hepes pH 7.4 (37°C), 2 mM $Mg(OAc)_2$, 150 mM NH_4OAc, 2 mM spermidine, 0.05 mM spermine, 4 mM ß-mercaptoethanol, and P RNA and *B. subtilis* P protein (RnpA) concentrations as indicated; the substrate concentration was 100 nM; 5'-endlabeled substrate was added in trace amounts (< 1 nM); k_{obs} is given in pmol substrate converted per pmol of P RNA per min; k_{rel} is defined as the ratio of k_{obs} obtained with the mutant versus wt holoenzyme under the respective conditions; values are based on at least four independent experiments.

REFERENCES

1. Wegscheid,B., Condon,C. and Hartmann,R.K. (2006) Type A and B RNase P RNAs are interchangeable *in vivo* despite substantial biophysical differences. *EMBO Rep.*, **7**, 411-417.

2. Mogk,A., Homuth,G., Scholz,C., Kim,L., Schmid,F.X. and Schumann,W. (1997) The GroE chaperonin machine is a major modulator of the CIRCE heat shock regulon of *Bacillus subtilis*. *EMBO J.*, **16**, 4579–4590.

3. Gößringer,M., Kretschmar-Kazemir Far,R. and Hartmann,R.K. (2006) Analysis of RNase P protein (*rnpA*) expression in *Bacillus subtilis* utilizing strains with suppressible *rnpA* expression. *J. Bacteriol.*, **188,** 6816-6823.

4. Smith,D., Burgin,A.B., Haas,E.S. and Pace,N.R. (1992) Influence of metal ions on the Ribonuclease P reaction. *J. Biol. Chem.*, **267**, 2429-2436.

5. Vioque,A., Arnez,J. and Altman,S. (1988) Protein-RNA interactions in the RNase P holoenzyme from *Escherichia coli*. *J. Mol. Biol.*, **202**, 835-848.

6. Busch,S., Kirsebom,L.A., Notbohm,H. and Hartmann,R.K. (2000) Differential role of the intermolecular base-pairs G292-C(75) and G293-C(74) in the reaction catalyzed by *Escherichia coli* RNase P RNA. *J. Mol. Biol.*, **299**, 941-951.