SUPPLEMENTARY INFORMATION

Construction of the *B. subtilis* conditional RNase P mutant strain SSB318

The *B. subtilis* conditional RNase P mutant (SSB318) was constructed as follows: a 251-nt PCR fragment, containing nucleotides +1 to +231 of the *rnpB* gene, was amplified using oligonucleotides HP499 (5'-ACC AAA GCT TGT TCT TAA CGT TCG GGT AAT CGC TG) and HP500 (5'-TCC AGG ATC CGT TTA CCG CGT TCC ACT CTC ACC ATT TC), digested with *Hind*III and *Bam*HI at the underlined sites and cloned in pMUTIN-4M (Vagner *et al.*, 1998) digested with the same enzymes. The resulting plasmid pMUTIN-*rnpB* was transformed into *B. subtilis* wild-type strain W168, selecting for erythromycin resistance in the presence of 1 mM IPTG. 95 out of 100 transformants tested showed IPTG-dependent growth, indicating correct insertion into the *rnpB* gene which is predicted to be essential. One of these transformants, SSB318, was retained for subsequent studies. In this strain, the chromosomal *rnpB* gene is under control of the IPTG-inducible *spac* promoter. In the absence of IPTG, cell growth becomes dependent on the expression of a complementing *rnpB* gene.

Plasmid cloning of *rnpB* genes for complementation analyses

For analyses in *E. coli* mutant strains, the complementing *E. coli rnpB* gene was expressed under control of the natural *E. coli rnpB* promoter from plasmid pSP64 as described (Hardt & Hartmann, 1996). For cloning of *B. subtilis rnpB* gene into the same plasmid, *B. subtilis rnpB* including its natural promoter and terminator was amplified from genomic *B. subtilis* DNA (strain W168) using primers 5'- GGC AGC AAG CTT TAT GAT TGA TCA C (including the native *Hind*III site upstream of *rnpB*) and 5'- CGC CCA AGC TTG TGT ATA CTT CTT CAT CGT ATC ACC CTG TC. The resulting PCR fragment was cloned in the *Hind*III site of

pSP64; the orientation of the *rnpB* gene was such that its 3'-portion was adjacent to the *Xba*I site in the pSP64 multi-cloning site.

B. subtilis rnpB was excised from this pSP64 construct via *Hind*III and inserted into pHY300 (obtained from Takara Shuzo Co., Kyoto, Japan) cut with the same enzymes for complementation in *B. subtilis* SSB318. For complementation of *E. coli rnpB* in *B. subtilis* SSB318, an *Nhe*I/*Hind*III fragment from plasmid pSP64 carrying the *E. coli rnpB* gene (Hardt and Hartmann, 1996) under control of the native *E. coli rnpB* promoter was cloned into pHY300 cut with *Xba*I and *Hind*III. *E. coli rnpB* under control of the *B. subtilis rnpB* promoter and terminator for complementation in *B. subtilis* SSB318 was constructed as follows. For amplification of the *B. subtilis* promoter, the primer pair 5'- GGC AGC AAG CTT TAT GAT TGA TCA C and 5'- ATG AAT TAT TAT ATA ACA ACT GAT TAC was used; the *B. subtilis* terminator was amplified using the primer pair 5'- ACA TTT AAA ATG ATG AAA ACA AGC and 5'- CGC CCA AGC TTG TGT ATA CTT CTT C; *E. coli rnpB* was amplified with primers 5'- GTA ATC AGT TGT TAT ATA ATA ATT CAT GAA GCT GAC CAG ACA GTC and 5'- GCT TGT TTT CAT CAT TTT AAA TGT AGG TGA AAC TGA AAC TGA CCG ATA AG, containing overlapping regions with the *B. subtilis rnpB* promoter and terminator, respectively. The *B. subtilis rnpB* promotor, the *E. coli* RNase P RNA-coding sequence and the *B. subtilis rnpB* terminator were combined by overlap extension PCR. This recombinant *E. coli rnpB* gene was cloned into the *Hind*III site of pHY300.

Construction of a strain containing *E. coli rnpB* with the native *B. subtilis* promoter and terminator integrated into the chromosome of *B. subtilis* SSB318

For construction of a strain containing *E. coli rnpB* with the native *B. subtilis* promoter and terminator integrated into the chromosome of *B. subtilis* SSB318, the *E. coli rnpB* fragment

was cloned into the *Hind*III site of pDG364, a vector suitable for chromosomal integration into the *amy*E locus of *B. subtilis* (Cutting and Vander-Horn, 1990). *E. coli rnpB* was amplified with primers 5'- GTA ATC AGT TGT TAT ATA ATA ATT CAT GAA GCT GAC CAG ACA GTC and 5'- GCT TGT TTT CAT CAT TTT AAA TGT AGG TGA AAC TGA AAC TGA CCG ATA AG, using plasmid pSP64 harbouring *E. coli rnpB* (Hardt and Hartmann, 1996) as template, and combined with *B. subtilis* promoter and terminator fragments by PCR as described above.

Transformation of *E. coli* complementation strains

Recombinant pSP64 plasmids (10 ng) were introduced in DW2/pDW160 by electroporation, using a Biorad GenePulser (1.8 kV; 5 ms, 50 μF, 100 □). After the pulse, 1 ml SOC medium (per liter: 20 g peptone, 5 g yeast extract, 0.6 g NaCl, 0.17 g KCl, 20 mM glucose, 10 mM $MgCl_2$, 10 mM MgSO4, pH 7.5) was added and cells were shaken at 30°C for 1 h. Cell suspensions were plated directly in appropriate dilution on LB agar plates containing 100 μg/ml ampicillin and 30 μg/ml chloramphenicol. The two sets of plates were incubated in parallel at 30°C or 43°C, respectivlely, for 20 to 40 h. For elimination of pDW160, DW2/pDW160 cells harboring a second complementation plasmid cells were grown at 43°C and then tested for loss of pDW160-encoded kanamycin resistance (50 μg/ml).

Transformation of *B. subtilis* complementation strain SSB318

B. subtilis SSB318 was grown overnight at 37°C in HS medium (Spizizen, 1958) without glucose, but complemented with 0.8% arginie, 0.04% histidine, 0.1% yeast extract 0.0005% L-tryptophan, 0.0005% L-phenylalanine and 0.02% casamino acids in the presence of 0.5 μg/ml erythromycin, 12.5 μg/ml lincomycin and 1 mM IPTG. Cultures were then diluted into

20 ml of LS medium (Spizizen minimal salts complemented with 0.1% yeast extract, 0.5 mM spermine, 2.5 mM $MgCl_2$, 0.01% casamino acids 0.0005% L-tryptophan and 0.0005% L-phenylalanine; Spizizen, 1958) to a starting OD_{600} of 0.05 - 0.1. Cells were then grown to an OD_{600} of 0.5 - 0.7 at 30°C under shaking. Approximately 1 μg DNA was added to 1 ml of this culture which was grown for another 1 to 2 h at 37°C. Cells were plated in parallel on LB agar plates with or without 1 mM IPTG and containing 0.5 μg/ml erythromycin, 12.5 μg/ml lincomycin and 30 μg/ml tetracycline (to select for the presence of pHY300). In cases of poor transformation efficiency, cells were first plated only in the presence of IPTG. For transformation of pDG364 derivatives, cells were plated on 0.5 μg/ml Erythromycin, 12.5 μg/ml lincomycin, 10 μg/ml chloramphenicol (encoded by pDG364) and 1 mM IPTG.

Determination of cell doubling time

E. coli DW2 cells harboring complementation plasmids were tested for the loss of kanamycin resistance (loss of pDW160). Kanamycin-sensitive and ampicillin- and chloramphenicol-resistant clones were grown overnight at 37°C and then diluted to a starting OD_{578} of 0.05 - 0.1 and grown at 37°C under aeration (180 rpm in a GFL Shaking Incubator 3033). In the case of complementation with *B. subtilis rnpB*, kanamycin-sensitive clones were verified by PCR to lack a chromosomal *E. coli rnpB* copy. *B. subtilis* cells were grown overnight at 37°C in the presence of the appropriate antibiotics and 1 mM IPTG. IPTG was then washed out by centrifugation and resuspension of the cell pellet in LB without IPTG, which was repeated; the final cell pellet was then resuspended in LB, adjusted to a starting OD_{578} of 0.05 - 0.1 in 50 mM LB and grown at 37°C without antibiotics at 180 rpm as above. After growth curve monitoring, samples were withdrawn from the cultures, diluted and plated in parallel on plates with and without antibiotics to exclude the loss of plasmids/resistance gene markers, both

types of plates containing 1 mM IPTG. Cell doubling times were derived from early exponential phases and reproduced in at least 3 independent experiments.

Construction of ptRNA^{Gly} templates encoding variants with C to G exchanges at position 74 or 75

Mutations G74 and G75 were introduced by the site-directed *Dpn*I mutagenesis method according to the manual provided with the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene); the primer pair for introduction of the G_{74} mutation was 5'-CCG TCT CCC GCT **G**CA GTC ACC GGA TGT GC and 5'-GCA CAT CCG GTG ACT G**C**A GCG GGA GAC GG; for introduction of the G_{75} mutation, 5'-CCG TCT CCC GCT C**G**A GTC ACC GGA TGT GC and 5'-GCA CAT CCG GTG ACT **C**GA GCG GGA GAC GG were used.

RT-PCR

To verify expression of *E. coli rnpB* from the *amy*E locus in the *B. subtilis* SSB318 background, cells were grown in parallel in the presence and absence of IPTG to an OD_{600} of 0.6 - 0.8. Total RNA from both cultures was prepared by the TRIzol method (Invitrogen) according to the protocol provided by the manufacturer, followed by DNase I treatment (Promega). RT-PCR was performed with the AccessQuick RT-PCR System (Promega) according to the manufacturer's instructions. Primers specific for *E. coli rnpB* were 5'- CTC ACT GGC TCA AGC AGC CT (5'-endlabeled in Fig. 3) and 5'- GAA GCT GAC CAG ACA GTC GC; primers specific for *B. subtilis rnpB* were 5'- TTC CGT TAA GAA GGT TCC CCT (5'-endlabeled in Fig. 3) and 5'- CTT AAC GTT CGG GTA ATC GC. For the

experiment in Fig. 3, RT-PCR reactions contained the normal amounts of unlabeled primers, and, in addition, trace amounts of the respective 5'-endlabeled primer.

References

Cutting SM, Vander-Horn PB (1990) Genetic analysis. In Harwood CR, Cutting SM (ed.), *Molecular biological methods for Bacillus* pp 53–54. John Wiley & Sons, Ltd., Chichester, UK

Hardt WD, Hartmann RK (1996) Mutational analysis of the joining regions flanking helix P18 in *E. coli* RNase P RNA. *J. Mol. Biol.* **259:** 422-433

Spizizen J (1958) Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proc. Natl. Acad. Sci. U.S.A.* **44:**1072-1078

Vagner V, Dervyn E, Ehrlich SD (1998) A vector for systematic gene inactivation in *Bacillus subtilis*. *Microbiology* **144:** 3097-3104