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Type A and B RNase P RNAs are interchangeable *in vivo* despite substantial biophysical differences

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We show that structural type A and B bacterial ribonuclease P (RNase P) RNAs can fully replace each other *in vivo* despite the many reported differences in their biogenesis, biochemical/biophysical properties and enzyme function *in vitro*. Our findings suggest that many of the reported idiosyncrasies of type A and B enzymes either do not reflect the *in vivo* situation or are not crucial for RNase P function *in vivo*, at least under standard growth conditions. The discrimination of mature tRNA by RNase P, so far thought to prevent product inhibition of the enzyme in the presence of a large cellular excess of mature tRNA relative to the precursor form, is apparently not crucial for RNase P function *in vivo*.

Keywords: RNase P; *in vivo* complementation studies; *Escherichia coli*; *Bacillus subtilis*
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INTRODUCTION

Ribonuclease P (RNase P) is an essential ribonucleoprotein enzyme responsible for the 5′-end maturation of transfer RNAs (Schön, 1999). Bacterial RNase P enzymes recognize the stacked acceptor stem/T-arm module of precursor tRNA (ptRNA) substrates (Harris & Christian, 2003, and references therein), and their RNA subunits have been shown to be catalytically active in the absence of the protein subunit (Guerrier-Takada *et al*, 1983). In bacteria, the RNA subunit (about 380 nt) forms a specific complex with a small basic protein of about 13 kDa, encoded by the *rnpA* gene. RNase P RNAs (P RNAs, encoded by the *rnpB* gene) from bacteria are subdivided into two distinct structural groups, termed type A (for 'ancestral') and type B (for '*Bacillus*'; Hall & Brown, 2001). *Escherichia coli* and *Bacillus subtilis* have been the principal model systems for type A and B RNase P RNAs, respectively. Although subunits of *E. coli* and *B. subtilis* RNase P enzymes have been shown to be interchangeable *in vitro* (Guerrier-Takada *et al*, 1983), more recent studies have indicated that the differences between the type A and B RNase P RNA architectures are associated with numerous differences in their biogenesis, biochemical/biophysical properties and enzyme function *in vitro*. There is evidence for autolytic processing of *B. subtilis* P RNA 5′ and 3′ ends after association with its protein subunit (Loria & Pan, 2000), whereas the *E. coli* P RNA precursor is processed by RNase E at its 3′ end and by an as yet unidentified ribonuclease at the 5′ end (Lundberg & Altman, 1995). *B. subtilis*, but not *E. coli*, RNase P was found to form a specific dimer consisting of two RNA and two protein subunits (Fang *et al*, 2001). Dimer formation is favoured in the absence of substrate, or in the presence of a tandem tRNA substrate, but disfavoured in the presence of a monomeric ptRNA (Barrera *et al*, 2002). The dimeric form of *B. subtilis* RNase P was further observed to bind to 30S ribosomal subunits, and this association has been proposed to modulate the enzyme's activity on potential, but as yet unidentified non-tRNA substrates (Barrera & Pan, 2004). The *B. subtilis* RNase P holoenzyme binds to ptRNA with a much higher affinity than mature tRNA (mtRNA), but this discrimination against mtRNA seems substantially attenuated in *E. coli* RNase P (Tallsjö & Kirsebom, 1993; Kurz *et al*, 1998). Indeed, a recent study reports a 1,600-fold preference of *B. subtilis* RNase P for ptRNA versus mtRNA, whereas this factor is reduced to 3 in the case of the *E. coli* holoenzyme (Buck *et al*, 2005). Finally, *E. coli* and *B. subtilis* P RNAs have distinct metal ion requirements (Warnecke *et al*, 1999).

In this report, we present the results of *in vivo* complementation experiments in RNase P mutant strains of *E. coli* and *B. subtilis*. The type B RNase P RNA of *B. subtilis* can functionally replace the type A RNA from *E. coli in vivo* and vice versa. Even a single copy of the *E. coli rnpB* gene inserted into the *B. subtilis* chromosome can fully rescue the growth defect caused by repression of endogenous *B. subtilis rnpB* gene expression.

RESULTS

In vivo complementation studies

The substantial differences between *E. coli* and *B. subtilis* RNase P outlined above raise the question as to whether the two RNAs can replace each other *in vivo*. An earlier study reported the successful

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Table 1 | Complementation of the *Escherichia coli* RNase P mutant strain by *Bacillus subtilis rnpB*

	DW2/pDW160		DW2 doubling time (min)*
RNase P RNA source	**30 °C**	**43 °C**	**37 °C**
pSP64-*EcrnpB*	+++	+++[‡]	75 ± 7
pSP64-*BsrnpB*	+++	+++[§]	64 ± 4
pSP64	+++	—	

Growth of mutant strains transformed with *E. coli* or *B. subtilis rnpB* was analysed on LB plates at the permissive (30 °C) and non-permissive (43 °C) temperature. RNase P, ribonuclease P; +++, good complementation; —, no colonies.
*Measured after elimination of pDW160.
[‡]Colonies at 43 °C were smaller than those at 30 °C; for growth at 43 °C, the phenotype of cells transformed with *E. coli rnpB* was set as the standard.
[§]Colonies at 43 °C were larger than those for cells transformed with *E. coli rnpB*, set as the standard.

complementation of an *E. coli rnpB* mutant strain by *B. subtilis rnpB*, but not by *rnpB* genes from the related bacteria *B. brevis* and *B. megaterium* (Waugh & Pace, 1990). Here, we describe cross-species complementation analyses in both *E. coli* and *B. subtilis*. In the *E. coli* mutant strain DW2/pDW160, the chromosomal *rnpB* gene is replaced with a chloramphenicol resistance cassette and a complementing *rnpB* gene is provided on a plasmid (pDW160) with a temperature-sensitive origin of replication (Waugh & Pace, 1990). Suppression of the conditionally lethal phenotype at 43 °C is achieved by expression of functional P RNA from a second compatible plasmid. For complementation in *B. subtilis*, we constructed a conditionally lethal mutant strain (SSB318), the endogenous *rnpB* expression of which is dependent on isopropyl-β-D-thiogalactoside (IPTG).

Complementation in *E. coli*

E. coli DW2 cells were transformed with the multicopy plasmid pSP64 encoding the *E. coli* or *B. subtilis rnpB* gene (pSP64-*EcrnpB* or pSP64-*BsrnpB*; Table 1). For growth curve monitoring, corresponding transformants were cured of plasmid pDW160 (loss of kanamycin resistance), and loss of the *E. coli rnpB* gene was verified by colony PCR for DW2 cells harbouring pSP64-*BsrnpB*. Consistent with the aforementioned study (Waugh & Pace, 1990), the *B. subtilis rnpB* gene rescued growth of the DW2 mutant strain at the non-permissive temperature of 43 °C (Table 1). Remarkably and for reasons as yet unknown, bacteria expressing *B. subtilis rnpB* formed larger colonies than cells transformed with the plasmid containing the homologous *E. coli rnpB* gene at 43 °C. This was also reflected in the faster growth rate of the cured strain in liquid cultures at 37 °C (64 versus 75 min doubling time in Luria-Bertani medium for DW2 cells transformed with pSP64-*BsrnpB* and pSP64-*EcrnpB*, respectively). Thus, *B. subtilis* P RNA can provide full RNase P function in *E. coli*, despite a tenfold lower affinity of this RNA for the *E. coli* RNase P protein (Day-Storms *et al*, 2004). It is possible that higher levels of expression of *B. subtilis rnpB* from the multicopy complementation plasmid compensate for the lower protein–RNA affinity.

Activity of hybrid holoenzymes from *E. coli*

E. coli DW2 bacteria expressing *E. coli* or *B. subtilis rnpB* from the complementation plasmid were cured of pDW160 and grown at 30 °C for partial purification of RNase P holoenzymes. Holoenzymes were tested under standard assay conditions (10 mM Mg^{2+}, 100 mM NH_4^+) for processing of $ptRNA^{Gly}$, as well as $ptRNAG_{74}$ and $ptRNAG_{75}$ variants (Fig 1), to assess cleavage efficiency and processing site selection following loss of the CCA interaction. Both enzymes cleaved $ptRNA^{Gly}$ at the canonical site (+1/−1). However, whereas the *E. coli* holoenzyme promoted substantial miscleavage of the mutant substrates at −1/−2, the hybrid enzyme containing *B. subtilis* P RNA did not significantly miscleave $ptRNAG_{74}$ or $ptRNAG_{75}$ (Fig 2A,B). These findings show that hybrid RNase P holoenzymes consisting of *B. subtilis* P RNA and *E. coli* P protein form stable complexes *in vivo* that can withstand biochemical purification. The results obtained with the mutant substrates further illustrate *in vitro* differences in substrate recognition between RNase P enzymes harbouring type A versus type B RNA subunits.

Complementation in *B. subtilis*

For complementation studies in *B. subtilis*, we constructed strain SSB318. This strain expresses the chromosomal *B. subtilis rnpB* gene under control of the *spac* promoter, and cell survival depends on the presence of IPTG. The conditionally lethal phenotype cannot be rescued by overexpression of the *B. subtilis* protein subunit (*rnpA*) from a plasmid (data not shown), indicating that small quantities of P RNA that may persist owing to *spac* promoter leakiness cannot be coaxed into activity by increased amounts of the protein subunit. SSB318 grows slower in the presence of 1 mM IPTG (doubling time 67 min; Table 2) than the wild-type strain W168 (57 min), suggesting that the fully induced *spac* promoter is not as strong as the native *B. subtilis rnpB* promoter and that P RNA is limiting for cell growth under these conditions. This is supported by the observation that the SSB318-derived strain SSB318*EcrnpB* (see below) has about 3.5-fold less *B. subtilis* P RNA in the presence of IPTG than W168 (Fig 3, lane 9 versus 17).

When expressed from a multicopy plasmid, under control of either its own or the *B. subtilis rnpB* promoter, the heterologous *rnpB* gene from *E. coli* fully rescued the conditionally lethal phenotype of SSB318 (Table 2). The generation time of the strain containing the *B. subtilis rnpB*-expressing plasmid was shorter (49 min) than that containing the *E. coli rnpB* genes (60 and 63 min for the constructs driven by the *E. coli* and *B. subtilis* promoters, respectively). Although this experiment might suggest that *E. coli rnpB* is not as efficient at restoring optimal growth as its *B. subtilis* counterpart, it is also possible that this was because of unrelated effects on plasmid copy number or stability. To eliminate complications of gene dosage from the plasmid-derived *rnpB* genes and differences in expression due to varying promoter

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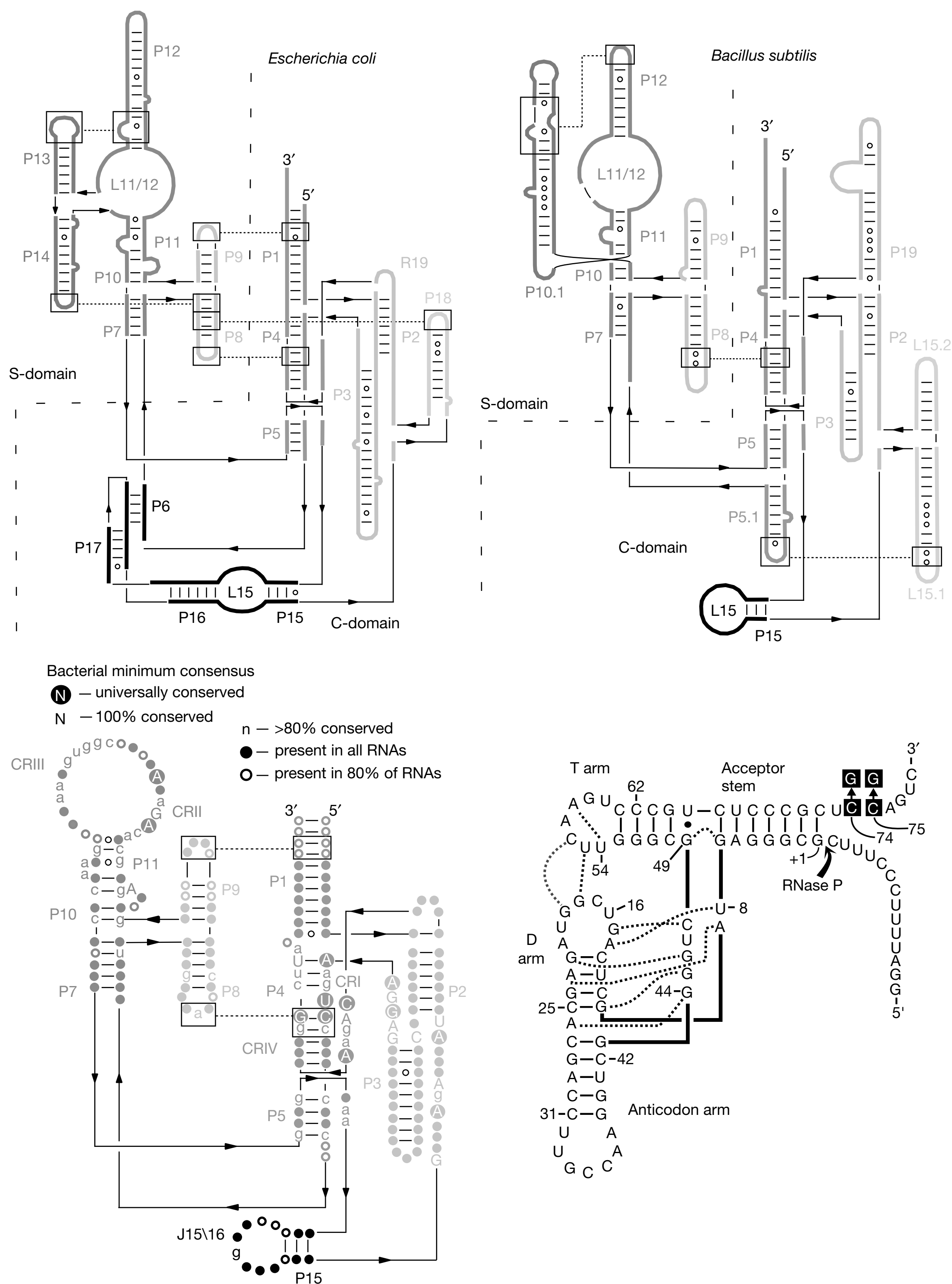

Fig 1 | Secondary structure illustrations (Tsai *et al*, 2003) of *Escherichia coli* (type A, top left) and *Bacillus subtilis* (type B, top right) ribonuclease P (RNase P) RNAs, and a phylogenetic minimum consensus RNase P RNA secondary structure (bottom left; Marquez *et al*, 2005); C-domain, catalytic domain; CR, conserved region; S-domain, specificity domain. Bottom right: *Thermus thermophilus* ptRNA^{Gly}; the arrow indicates the canonical RNase P cleavage site between nucleotides −1 and +1; the point mutations in 3′-CCA of ptRNA^{Gly} are highlighted by black squares; tertiary interactions inferred from the crystal structure of yeast tRNA^{Phe} are indicated by dotted lines (adapted from Heide *et al*, 2001).

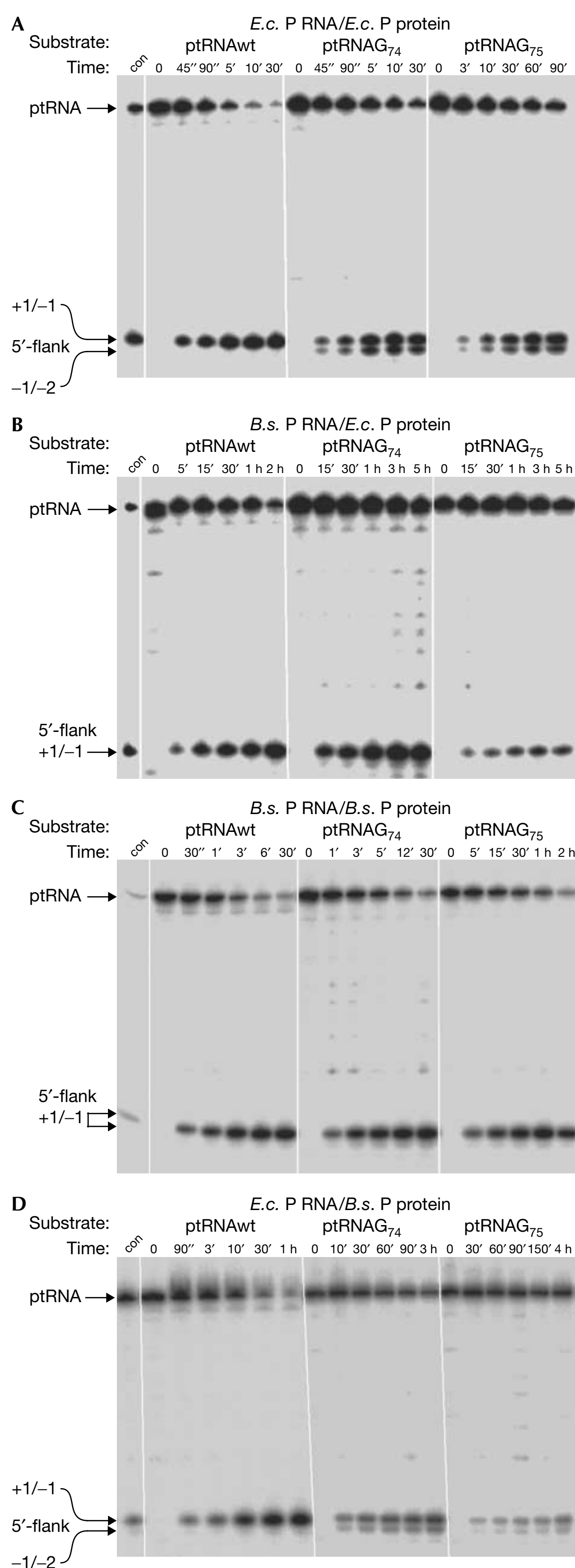

Fig 2 | Processing and cleavage site selection of hybrid ribonuclease P (RNase P) holoenzymes isolated from *Escherichia coli* and *Bacillus subtilis* complementation strains. (**A,B**) RNase P partially purified from *E. coli* DW2 or (**C,D**) *B. subtilis* SSB318; holoenzymes contained *E. coli* P RNA in (**A,D**) or *B. subtilis* P RNA in (**B,C**). con, control cleavage by *E. coli* RNase P RNA. For details, see Methods. 5′-Cleavage products are indicated on the left (canonical cleavage site at $+1/-1$, miscleavage at $-1/-2$). Although the amount of extract added to processing assays was equalized in (**A–D**), based on absorption at 260 nm, holoenzyme concentrations are probably identical only within one panel; *E.c.*, *E. coli*; *B.s.*, *B. subtilis*.

strength, we constructed strain SSB318*EcrnpB*, which carries a single copy of the *E. coli rnpB* gene under control of the *B. subtilis rnpB* promoter in the *amyE* locus on the *B. subtilis* chromosome. Constitutive expression of *E. coli rnpB* in this strain was confirmed by radioactive reverse transcription–PCR (RT–PCR; Fig 3, lanes 7,11). The single-copy chromosomal *E. coli rnpB* gene efficiently rescued the lethal phenotype in the absence of IPTG, even when the *lac* repressor was overproduced from plasmid pMAP65 to tighten repression of the *spac* promoter (Table 2). The growth rates of SSB318*EcrnpB* and SSB318*EcrnpB* pMAP65 (57 and 64 min, respectively) were indistinguishable from those of the wild-type strains W168 and W168 pMAP65 (56 and 65 min, respectively), suggesting that *E. coli* P RNA not only carries out all the essential RNase P functions in the heterologous *B. subtilis* host, but is also highly efficient at catalysing any of its potential minor functions (see below). It is also interesting to note that expression of both P RNA types in the same cell, whether in single copy or on a plasmid, did not pose any problem for *B. subtilis* growth (Table 2).

In experiments similar to those performed with the *E. coli* DW2 strain, we partially purified holoenzymes from *B. subtilis* SSB318 transformed with *E. coli* or *B. subtilis rnpB* and grown in the absence of IPTG. In agreement with the complementary data in Fig 2A,B, the *B. subtilis* holoenzyme did not significantly miscleave ptRNAG$_{74}$ or ptRNAG$_{75}$, whereas the hybrid enzyme containing *E. coli* P RNA promoted substantial miscleavage of the mutant substrates (Fig 2C,D).

DISCUSSION

Our study shows that type A and B RNase P RNAs are interchangeable *in vivo* under standard growth conditions, indicating that the hybrid enzymes can carry out all essential cellular functions of RNase P. These findings are surprising for several reasons. First, the lower stability of hybrid holoenzyme complexes (Day-Storms *et al*, 2004) evidently does not pose a problem for holoenzyme assembly *in vivo*, as a single *E. coli rnpB* copy can provide full RNase P function in *B. subtilis* SSB318. Second, Buck *et al* (2005) determined that the native *B. subtilis* holoenzyme has a 1,600-fold preference for ptRNA compared with that for mtRNA, whereas this selectivity is reduced twofold for the hybrid enzyme containing *E. coli* P RNA. Our results show that this change in the relative affinities for ptRNA and mtRNA does not abrogate RNase P function *in vivo*. Third, the *E. coli* protein reduces the Mg^{2+} concentration requirement for tertiary structure formation of P RNA and increases the melting temperature at physiologically relevant Mg^{2+} concentrations (Buck *et al*, 2005). In contrast, the *B. subtilis* protein does not exert such stabilizing effects on its cognate or *E. coli* P RNA (Buck *et al*, 2005). As *E. coli* P RNA can

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Table 2|Complementation of *Bacillus subtilis* SSB318 RNase P mutant strains by *Escherichia coli rnpB* (cell doubling times given in minutes)

***rnpB* gene source**	**Promoter***	**IPTG**	**No IPTG**
B. subtilis W168 (wild type)			
W168	*B.s.* native	57±2	56±2
W168+pMAP65[‡]	*B.s.* native	62±2	65±2
B. subtilis SSB318 (Pspac:rnpB)			
SSB318	*spac*	67±3	—
SSB318+pMAP65[‡]	*spac*	81±6	—
Multicopy			
pHY300 (empty plasmid)	None	69±4	—
pHY300-*BsrnpB*	*B.s.* native	49±1	49±2
pHY300-*EcrnpB*	*E.c.* native	52±2	60±2
pHY300-*EcrnpB*	*B.s.* native	58±3	63±3
Single copy			
pDG364[§]	None	63±3	—
SSB318*EcrnpB*[‖]	*B.s.* native	52±1	57±1
SSB318*EcrnpB*+pMAP65[‡]	*B.s.* native	56±2	64±4

RNase P, ribonuclease P; IPTG, isopropyl-β-D-thiogalactoside; —: no growth at all; for determination of cell doubling times, see the supplementary information online.
*Promoter used for the respective *rnpB* gene; *B.s.* native and *E.c.* native, native *rnpB* promoters from *B. subtilis* and *E. coli*, respectively.
[‡]Plasmid pMAP65 (pUB110 *lacI*; Petit *et al*, 1998) was used to overexpress the *lac* repressor to fully silence expression from the *spac* promoter in strain SSB318.
[§]Vector used for chromosomal integration into the *amyE* locus.
[‖]*E. coli rnpB* integrated into the *amyE* locus under control of the native *B. subtilis rnpB* promoter and terminator.

provide RNase P function in *B. subtilis* (Table 2), these differences in their biochemical and biophysical properties apparently do not have important consequences for hybrid RNase P function *in vivo*.

B. subtilis RNase P can form symmetric dimers consisting of two RNA and two protein subunits, whereas *E. coli* P RNA tends to form aggregates (Fang *et al*, 2001). Buck *et al* (2005) reinvestigated dimerization by *B. subtilis* and *E. coli* RNase P, and observed weak dimer formation by the *E. coli* holoenzyme in addition to higher order aggregation. The K_D of dimer formation was about 50–100 nM for *B. subtilis* and 500–1000 nM for *E. coli* RNase P, with the dimerization properties mainly determined by the type of P RNA subunit (Buck *et al*, 2005). Unlike the *B. subtilis* enzyme, dimers containing *E. coli* P RNA readily dissociated to monomers in the presence of mtRNA, which correlated with the high-affinity binding of mtRNA by holoenzymes containing *E. coli* P RNA. As the mtRNA concentration in an *E. coli* cell is estimated to be 500 μM in the presence of 2 μM (Hansen *et al*, 2001) or lower (Buck *et al*, 2005) concentrations of cellular RNase P, the biological relevance of dimer formation by holoenzymes containing *E. coli* P RNA is questionable. Our observation that *E. coli* P RNA functions in *B. subtilis* RNase P mutant strains also makes it unlikely that RNase P dimer formation exerts an important regulatory role in *B. subtilis*.

The maturation pathways of the primary P RNA transcripts differ in *E. coli* and *B. subtilis*. The *E. coli* pathway requires RNase E, an essential enzyme that is not found in *B. subtilis*, whereas processing of *B. subtilis* P RNA may be at least partly autocatalytic. The results presented here indicate that not only are the P RNAs interchangeable, but so too are their maturation pathways, assuming the P RNA primary transcripts have to undergo processing for proper function.

Finally, *E. coli* RNase P is known to process several non-tRNA substrates *in vivo* (Li & Altman, 2003), whereas non-tRNA substrates have not yet been identified in *B. subtilis*. As *B. subtilis* P RNA provided full RNase P function in *E. coli*, hybrid holoenzymes containing this RNA subunit are able to process at least the essential non-tRNA substrates in *E. coli*, such as 4.5S RNA (Brown & Fournier, 1984). The fact that *B. subtilis* strains expressing single-copy *E. coli* or *B. subtilis rnpB* genes have the same doubling time suggests that, should *B. subtilis* RNase P also have alternative functions, the *E. coli* hybrid enzyme is highly efficient at performing them.

In vivo analyses of the kind reported here provide a framework to test the functional relevance of the numerous biochemical, biophysical and enzymatic differences that have been observed between bacterial type A and B RNase P enzymes. Our results show that, despite these differences, the heterologous hybrid enzymes are capable of carrying out the essential functions of RNase P *in vivo*, although potential defects under particular stress conditions cannot be excluded at present. This poses the question

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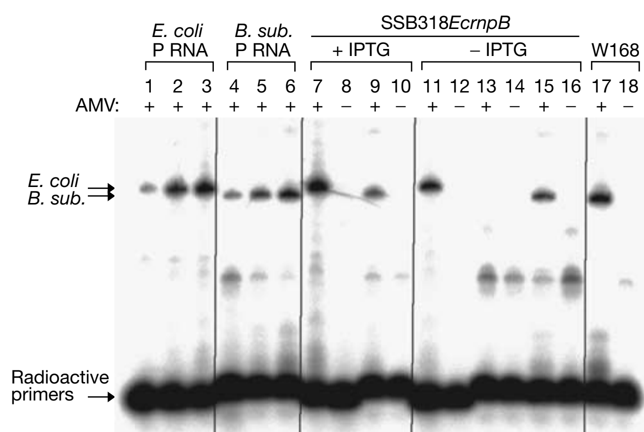

Fig 3 | Radioactive reverse transcription PCR (RT–PCR) analysis of the SSB318-derived strain, SSB318*EcrnpB*. PCR products were analysed on an 8% polyacrylamide/8 M urea gel; lanes 1–3: 0.2, 1 or 5 pg, respectively, of *in vitro*-transcribed *Escherichia coli* ribonuclease P RNA (P RNA) added to 0.5 μg *Bacillus subtilis* W168 (wild type) total cellular RNA; lanes 4–6: 0.2, 1 or 5 pg, respectively, of *in vitro*-transcribed *B. subtilis* P RNA added to 0.5 μg of total cellular RNA from *E. coli* DW2 grown at 30 °C; lanes 7–16: total RNA from SSB318*EcrnpB* grown in the presence (lanes 7–10) or absence (lanes 11–16) of 1 mM isopropyl-β-D-thiogalactoside (IPTG); lanes 7,8,11,12: RT–PCR with primers specific to *E. coli* P RNA; lanes 9,10,13–16: RT–PCR with primers specific to *B. subtilis* P RNA; lanes 15,16: the total RNA preparation was supplemented with 1 pg of *in vitro*-transcribed *B. subtilis* P RNA; lanes 17,18: total RNA from the *B. subtilis* W168 wild-type strain using primers specific for *B. subtilis* P RNA. AMV + or – indicates the presence (+) or absence (–) of reverse transcriptase. For details on RT–PCR, see the supplementary information online. Lanes 1–6 document that the amount of PCR product is sensitive to RNA template concentration (arrows on the left indicate the *E. coli* and *B. subtilis* P RNA-specific amplification products); lane 15 shows that the RNA preparation (–IPTG) does not inhibit the RT–PCR reaction with *B. subtilis* P RNA-specific primers. For all RT–PCR reactions, the total amount of RNA was 0.5 μg.

as to which conserved features of bacterial RNase P RNA and protein subunits are essential for *in vivo* function. Both the *E. coli* and *B. subtilis* P proteins increase the affinity of P RNA for ptRNA by factors of 700–1400 for hybrid and native enzymes containing *E. coli* P RNA, and 13,000–33,000 for those containing *B. subtilis* P RNA (Buck *et al*, 2005). As the *E. coli* P RNA/*B. subtilis* P protein complex permitted rescue of the mutant phenotype of *B. subtilis* strain SSB318, we can conclude that a 700-fold increase in ptRNA affinity conferred by the protein subunit is sufficient to support *in vivo* function. This increase in affinity for ptRNA is linked to an increase in the affinity for key metal ions involved in ptRNA binding and catalysis (Kurz & Fierke, 2002), probably achieved by the protein's stabilization of conserved local structure in the catalytic core of type A and B RNase P RNAs. These central functions are evidently conserved across bacterial species barriers. In the course of evolution, type A and B RNase P enzymes have developed RNA subunits with different architecture and individually tailored protein subunits, optimized for their specific cellular milieu. It is thus fascinating to observe that these RNase P enzymes still retain their biological function when their RNA subunit is exchanged with one representing a different architectural type.

METHODS

Strains. *E. coli* strain DW2/pDW160 was used for complementation studies (see text). For construction of the *B. subtilis* conditional RNase P mutant strain SSB318, its derivative harbouring a single copy of *E. coli rnpB*, as well as complementation plasmids, see the supplementary information online.

***In vitro* transcription and 5′-end labelling.** Runoff transcription with bacteriophage T7 RNA polymerase and 5′-end labelling was performed as described previously (Busch *et al*, 2000). The ptRNA^{Gly} substrate, and G74 and G75 variants (Fig 1; for construction, see the supplementary information online) were transcribed from plasmid pSBpt3′HH, and *B. subtilis* and *E. coli* P RNA from plasmids pDW66 and pJA2′, respectively (Busch *et al*, 2000).

Partial purification of RNase P. Cells resuspended in about 4 ml of buffer A (60 mM NH_4Cl, 10 mM $Mg[OAc]_2$, 6 mM dithiothreitol, 50 mM Tris–HCl, pH 7.2–7.5) per 600 mg cell pellet were lysed by sonication (Branson Sonifier 250, output 20, duty cycle 30–40%, 10 min on ice), followed by centrifugation for 45 min at 4 °C and 10,000*g*. For the preparation of DEAE fractions, the supernatant was loaded onto a DEAE fast-flow Sepharose column (Aekta basic, GE Healthcare Europe, Munich, Germany). Elution was carried out by applying a continuous NH_4Cl gradient from 60 to 550 mM NH_4Cl. Active fractions eluted at about 300–400 mM NH_4Cl.

Processing assays. For RNase P-catalysed processing of *Thermus thermophilus* ptRNA^{Gly} (and G_{74} or G_{75} variants; Fig 1), active DEAE fractions (0.25–1.0 μl; equal amounts based on A_{260} measurements) were incubated in buffer A (10 mM $Mg[OAc]_2$, 6 mM dithiothreitol, 50 mM Tris–HCl, pH 7.5) containing 100 mM NH_4Cl for 10 min at 37 °C. Trace amounts (<1 nM) of 5′-end-labelled substrate were preincubated under the same conditions for 5 min at 55 °C and 25 min at 37 °C. Processing reactions were started by combining enzyme and substrate solutions and assayed at 37 °C. For control cleavage by *E. coli* RNase P RNA (Fig 2), trace amounts (<1 nM) of 5′-end-labelled ptRNA^{Gly} were incubated with 5 μM *E. coli* RNase P RNA for 5 s at 37 °C in 50 mM PIPES, 0.1 M $Mg[OAc]_2$ and 1 M NH_4OAc (pH 7.0 at 37 °C).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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