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Essential is Not Irreplaceable: Fitness Dynamics of Experimental *E. coli* RNase P RNA Heterologous Replacement

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Abstract While critical cellular components—such as the RNA moiety of bacterial ribonuclease P—can sometimes be replaced with a highly divergent homolog, the cellular response to such perturbations is often unexpectedly complex. RNase P is a ubiquitous and essential ribonucleoprotein involved in the processing of multiple RNA substrates, including tRNAs, small non-coding RNAs and intergenic operons. In Bacteria, RNase P RNAs have been subdivided—based on their secondary and tertiary structures—into two major groups (A and B), each with a distinct phylogenetic distribution. Despite the vast phylogenetic and structural gap that separates the two RNase P RNA classes, previous work suggested their interchangeability. Here, we explore in detail the functional and fitness consequences of replacing the endogenous Type-A *Escherichia coli* RNase P RNA with a Type-B homolog derived from *Bacillus subtilis*, and show that *E. coli* cells forced to survive with a chimeric RNase P as their sole source of RNase P activity exhibit extremely variable responses. The chimeric RNase P alters growth rates—used here as an indirect measure of fitness—in unpredictable ways, ranging from 3- to 20-fold reductions in maximal growth rate. The transcriptional behavior of cells harboring the chimeric RNAse P is also perturbed, affecting the levels of at least 79 different transcripts. Such transcriptional plasticity represents an important mechanism of transient adaptation which, when coupled with the emergence and eventual fixation of compensatory mutations, enables the cells to overcome the disruption of this tightly coevolving ribonucleoprotein.

Keywords RNase P · Ribozyme · tRNA processing · *rnpB* · M1 RNA

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Introduction

The bacterial ribonucleoprotein RNase P is a central player in the control and regulation of bacterial growth (Bourgaize and Fournier 1987; Cherayil et al. 1987; Panagiotidis et al. 1992; Dong et al. 1996; Kim et al. 1998). This versatile catalyst is responsible for the processing of an ever-expanding catalogue of cellular RNAs, including the 5′ terminus of tRNA, tmRNA, 4.5S RNA, phage C4 RNA as well as a number of polycistronic operons (Altman and Smith 1971; Peck-Miller and Altman 1991; Komine et al. 1994; Hartmann et al. 1995; Alifano et al. 1994; Li and Altman 2003). As the first ribozyme described, the RNA component (M1 RNA) of this ribonucleoprotein has attracted deserved notoriety (Guerrier-Takada et al. 1983; Altman 2011). Considerable work has gone into understanding its structure, function, regulation, and evolution (e.g., Masquida and Westhof 2011; Krasilnikov et al. 2003; Kazantsev and Pace 2006; Krivenko et al. 2002; Torres-Larios et al. 2006; Jarrous and Gopalan 2010).

In Bacteria, RNase P RNAs are classified, based on the conservation of secondary and tertiary structures, into two major groups (A and B) (Esakova and Krasilnikov 2010) (Fig. 1). Type-A RNase P RNA is found in the majority of bacterial species; Type-B is comparatively rare and only found in certain low G+C Gram-positive bacterial species (Hall and Brown 2001). Both the structural contrasts and the phylogenetic distributions of these two major RNase P RNA classes suggest an ancient bifurcation: few, if any, "intermediate" forms have been uncovered. The ability of a Type-B RNase P RNA to complement a deletion of its Type-A counterpart in *E. coli* was thus unexpected (Lawrence et al. 1987; Waugh and Pace 1990; Wegscheid et al. 2006). This study undertakes a detailed experimental exploration of the consequences of the heterologous replacement of the Type-A M1 RNA in *E. coli*. To do so, we have deleted the chromosomal version of the *rnpB* gene encoding M1 RNA, all the while keeping the cell alive through the use of a rescue plasmid. This rescue plasmid was, in turn, displaced by a selection plasmid harboring the heterologous Type-B version of the *rnpB* gene. We then closely monitored the growth of the resulting cell lines, now entirely dependent on the heterologous *rnpB* transcript—and thus on a chimeric RNase P—for survival.

Here, we examine the effects of heterologous replacement on the viability and growth of the recipient *E. coli* cells. We use maximal growth rate as a proxy for fitness; a well-established practice for both bacterial (Guo et al. 2012; Pope et al. 2009; van Opijnen and Camilli 2013) and viral (Bull et al. 1997) fitness assays. This quantitative information begins to illuminate the topography of the underlying adaptive landscape. Coupled to an exploration of genome-wide changes in transcription triggered by the heterologous replacement, these results provide a closer look at the mechanisms that may enable bacterial cells to cope with severe perturbations. Our results may also provide a window into the genetic and evolutionary aftermath of horizontal gene transfer across important phylogenetic gaps.

Results

In Vivo *rnpB* Complementation System

To investigate the fitness effects of *rnpB* complementation, we developed a system based on FLP recombinase activity (Meyer-Leon et al. 1984; Hoang et al. 1998) that allows us to replace the endogenous *rnpB* gene with any *rnpB* ortholog or with a synthetic version. Because *rnpB* is an essential gene, an alternative source of *rnpB* expression is required to rescue the lethal deletion. In our *rnpB* null strain [(DW2/pRescue), derived from the DW2 strain

A Secondary structure
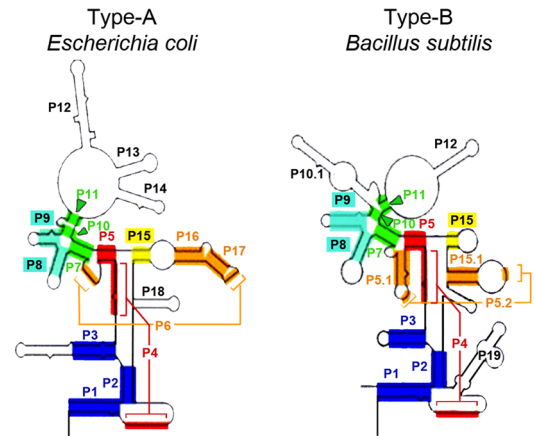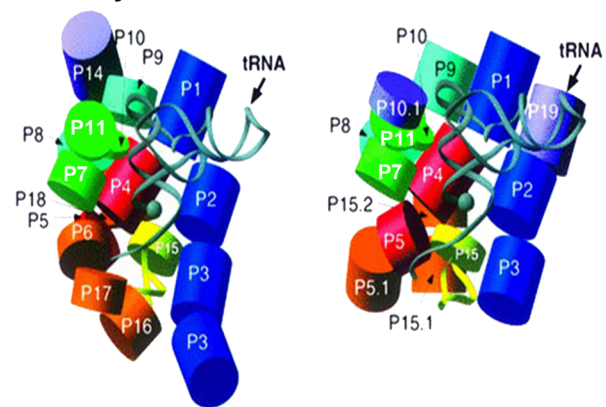

B Tertiary structure

Fig. 1 Models of secondary and tertiary structures of *E. coli* and *B. subtilis* RNase P RNAs. **a** Secondary structures of *E. coli* and *B. subtilis* RNase P RNAs, helices P1-P17 are labeled and colored by helical domain. **b** Proposed tertiary structures of *E. coli* (*left*) and *B. subtilis* (*right*) RNase P RNA–tRNA complexes. The cyan ribbon represents a tRNA, with the 5′-phosphate site shown as a *grey sphere*. *Yellow loop* represents the CCA-binding site in RNase P RNA. Adapted from (Chen et al. 1998), reproduced with permission from EMBO (Color figure online)

described in (Waugh and Pace 1990)] the chromosomal *rnpB* gene is replaced by a chloramphenicol resistance marker, and cell viability is maintained by a plasmid (pRescue) that harbors the *E. coli rnpB* gene driven by *E. coli rnpB* promoters and flanked by FLP recognition target (FRT) sites in identical orientation (Supplementary Figs. 1, 2; Supplementary Table 1).

The key strategy of our FLP-mediated complementation system involves abolishing the expression of *E. coli rnpB* (originating from the pRescue plasmid) and replacing it with that of an *rnpB* homolog of our choosing, still driven by *E. coli rnpB* promoters. This was accomplished by inserting a second plasmid (pFLP) that included a selectable antibiotic marker, a constitutively expressed FLP recombinase, and an *rnpB* gene [either *E. coli rnpB* for the
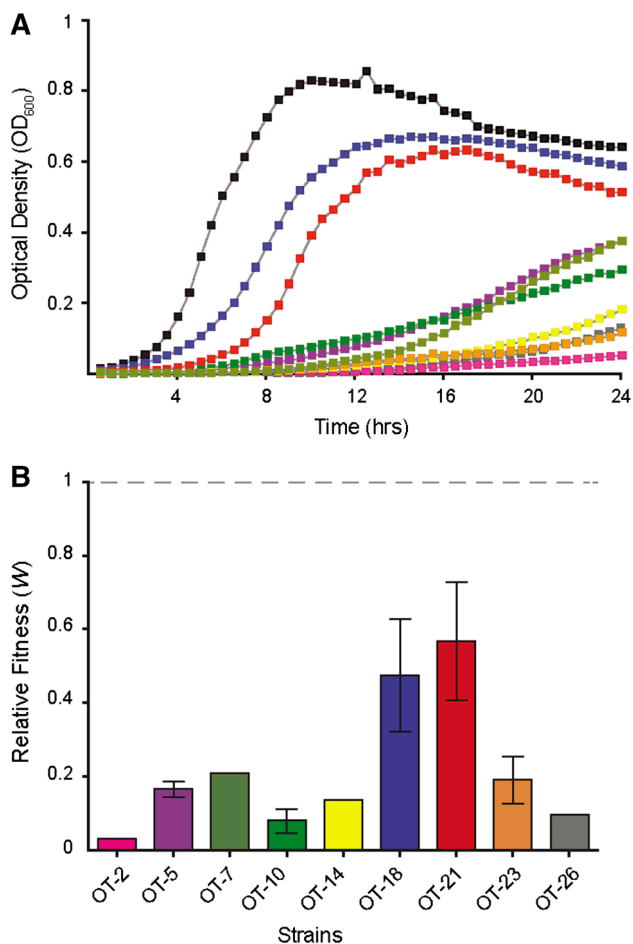
Fig. 2 In vivo growth of DW2/pFLP-Bs original transformants.
a Growth rate in liquid culture measured by optical density (OD_{600})
readings (*squares*) taken at 30 min intervals over 24 h of one
representative control strain (*black*) and 9 representative original
transformants (OT) DW2/pFLP-Bs transformants: OT-2 (*pink*), OT-5
(*purple*), OT-7 (*olive*), OT-10 (*green*), OT-14 (*yellow*), OT-18 (*blue*),
OT-21 (*red*), OT-23 (*orange*), OT-26 (*gray*). Data shown was
collected in a single experiment; we show the average growth rate for
each transformant line measured across three replicates. **b** Growth
rates expressed as relative fitness (*W*), where control strain
fitness = 1 (*dotted line*). *Colors* are the same as in (**a**) and represent
W from a single experiment (for OT-2, OT-7, OT-14, OT-26), mean
W from two (for OT-5, OT-21, OT-23) and three (for OT-10, OT-18)
experiments; *error bars* show ±SD. See Supplemental Table S5 for
details (Color figure online)

control strain (pFLP-Ec) or *Bacillus subtilis rnpB* for our
test strain (pFLP-Bs)] under the control of *E. coli rnpB*
promoters (Supplementary Fig. 2b; Supplementary
Table 1). When our deletion strains take up the pFLP
plasmid, the consequent FLP recombinase activity results
in the linearization and degradation of pRescue (Supple-
mentary Fig. 1c, d).

Because we sought to capture and quantify the fitness
costs that result from *rnpB* complementation, we charac-
terized a set of transformants, chosen at random, from each
of our transformation experiments. We defined a

transformation event to be the transformation with a
specified plasmid of a single aliquot containing an isogenic
population of DW2/pRescue cells. Accordingly, we trans-
formed one vial of isogenic DW2/pRescue cells with the
pFLP-Bs plasmid to create the DW2/pFLP-Bs strain, and in
parallel, transformed another vial of the same strain with
pFLP-Ec as a positive transformation control to create the
DW2/pFLP-Ec strain. Successful transformation was con-
firmed by observing the expected antibiotic resistance
profile (Supplementary Table 1), by verifying that no
E. coli rnpB RNA could be detected by quantitative RT-
PCR (Supplementary Fig. 4) and by performing a diag-
nostic digest of plasmids.

Fitness Assays

We quantified the costs of relying solely on *B. subtilis rnpB*
for RNase P activity using growth rate in liquid culture
under nutrient rich conditions as a proxy for fitness (Dong
et al. 1996; Wegscheid et al. 2006). We compared, over a
24 h time course, averaged absorbance measurements at
30 min intervals (Fig. 2a; Supplementary Fig. 3). The
maximal growth rate for each strain was estimated as the
maximal slope during the exponential growth phase; these
values were subsequently used to calculate relative fitness.
For each fitness assay, the maximal slope for DW2/pFLP-
Ec was normalized to 1 and relative fitnesses were calcu-
lated accordingly (Supplementary Table 5).

We began by investigating the fitness effects of
placing the endogenous *E. coli rnpB* gene and its asso-
ciated promoters and terminators on a plasmid. To do so,
we compared the growth rate of a wild type strain (BZB
1011) harboring the *rnpB* gene in its normal chromo-
somal position to that of our *rnpB*-Δ strains in which the
rnpB gene is located on a plasmid (DW2/pRescue and
DW2/pFLP-Ec). The growth curves suggest no discern-
ible difference in the growth profiles of these strains
under our experimental conditions (Supplementary
Fig. 3). We thus used DW2/pFLP-Ec as a proxy for wild
type ("control strain") in all subsequent growth curve
experiments.

By measuring the growth rate of DW2/pFLP-Bs trans-
formants in detail, we hoped to capture the precise fitness
costs of heterologous replacement of the RNase P RNA
component. We anticipated, based on previous reports
(Wegscheid et al. 2006), that the DW2/pFLP-Bs transfor-
mants would show reduced fitness relative to the control
strain as a result of their reliance on a foreign, albeit
functional, *rnpB* gene for RNase P activity (Wegscheid and
Hartmann 2007; Haga et al. 2004; Pomeranz, Krummel and
Altman 1999; Yan and Francklyn 1994). All characterized
DW2/pFLP-Bs transformants grew more slowly than did
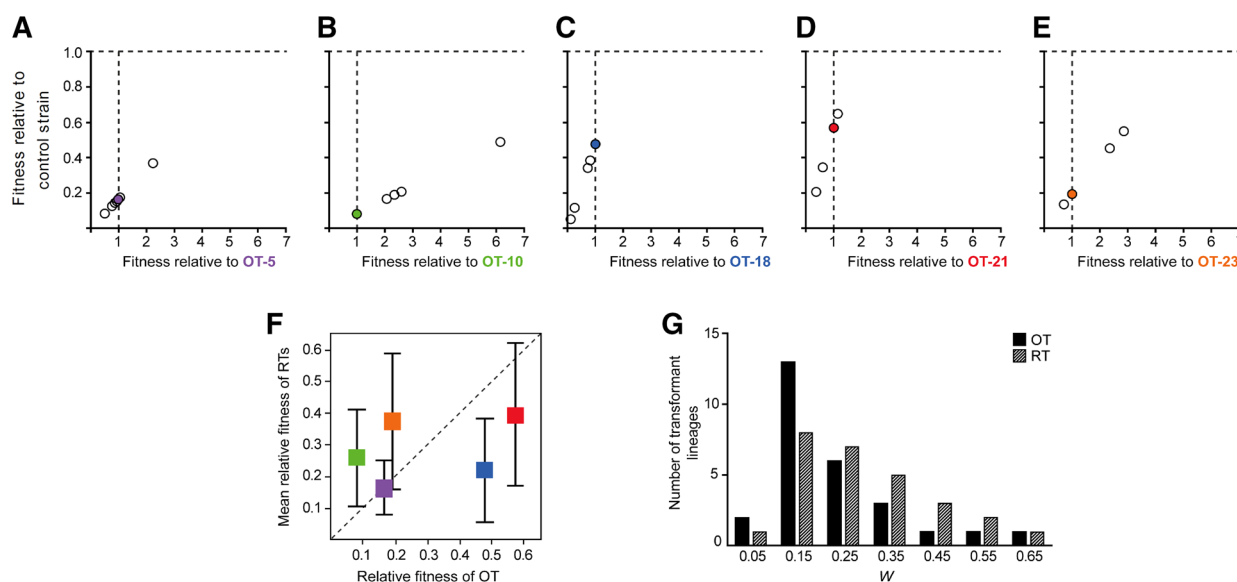their control strain counterparts. What we had not

Fig. 3 Comparison of RT fitness relative to plasmid donor OT lineage and to the control strain. The fitness of RT lineages (*open circles*) normalized to their corresponding plasmid donor OT lineage (*filled circles*) (x-axis *dotted line*, $W = 1$) and to the control strain (y-axis *dotted line*, $W = 1$) is depicted in (**a–e**) for lineages derived from OT-5, OT-10, OT-18, OT-21, and OT-23, respectively. Panel (**f**) shows the correlation (*filled squares*) between the relative fitness of OT lineages and the mean relative fitness of their RT lineages, expressed as fitness relative to control strain, *error bars* show ±SD. The *dashed diagonal line* represents the expected correlation between OT fitness and corresponding RT mean fitness, if these values were perfectly correlated. **g** Distribution of W collected from 3 independent fitness assays for OT and RT groups. Bin ranges mark 0.1 value intervals with first and last bins as $(0 < W < 0.05)$ and $(0.55 < W < 0.65)$, respectively. All colors are as in Fig. 2 (Color figure online)

expected, however, was the surprising variability in the fitness consequences of the heterologous replacement (Figs. 2, 3g).

A number of factors could account for the range of growth rates we observed in the DW2/pFLP-Bs transformants. These include compensatory mutations in the *B. subtilis rnpB* gene, its promoters, or the chromosomal gene (*rnpA*) encoding the protein moiety of the RNase P holoenzyme, or in other regions of the plasmid or chromosome unlinked to the *rnpA* and *rnpB* genes. Given the magnitude of the perturbation, we are undoubtedly selecting for compensatory mutations that mitigate the functional consequences of the chimeric RNase P. We note, however, that the heterologous *rnpB* is being introduced into an isogenic *E. coli* line that has undergone only a limited number of doublings, and is thus unlikely to have accumulated a large number of possible compensatory mutations. Instead, the range of fitnesses we observe could reflect the interaction between available compensatory mutations and non-heritable compensatory changes in the transcription of one or more genes—changes that contribute to alleviating the deleterious effects of the heterologous replacement.

Testing Fitness Heritability in a Second Series of Transformations

We were able to rule out compensatory mutations in the *B. subtilis rnpB* gene, the *rnpB* promoter region and the chromosomal *rnpA* gene for all DW2/pFLP-Bs transformants tested by directly sequencing those regions: no sequence changes were found. In order to investigate the possibility that mutations had arisen in the bacterial chromosome or regions of pFLP-Bs plasmid that we had not yet sequenced, we focused on the heritability of the observed fitness.

Using pFLP-Bs plasmids extracted from previously characterized DW2/pFLP-Bs transformants, we performed a second series of transformations into a fresh stock of recipient DW2/pRescue competent cells. We hypothesized that if the growth rates seen in the first round of transformants (referred to as *original transformants*, OT) resulted from uncharacterized compensatory changes in the chromosome, such changes—and their resulting fitness effects—would not be carried over into the *re-transformants* (RT). Transformations to create RT strains were carried out exactly as described above (Supplementary Fig. 1), with one modification: the source of the pFLP-Bs plasmid was a confirmed OT DW2/pFLP-Bs transformant that had undergone the original fitness assays. Accordingly, each of the original DW2/pFLP-Bs transformant lineages 'donated' a plasmid which was used to create the second generation of DW2/pFLP-Bs transformants. The system we used to catalogue RT strains is illustrated in the following example: if OT-5 was a plasmid donor, corresponding RT strains were labeled RT-5-1, RT-5-2, RT-5-3, and so on.

We examined the growth rates of these RT strains as previously described, with a particular interest in the extent to which the growth rate of the OT donor lineage matches the growth rate of its corresponding RT lineages. Figure 3a–e shows the fitnesses of the OT donor lineage and of its RT descendants relative to the fitness of the control strain and of the OT strain. Panels (a–e) reveal that the fitness of RT lineages does not faithfully replicate that of their OT progenitors. Furthermore, the direction of fitness change that results from the second transformation cannot be predicted, as we observed both increases and decreases in the fitness of RT lineages relative to that of the progenitor strain. Only in the case of strain OT-10 do all of the RT lineages show an increase in relative fitness. This may reflect the low initial fitness of the OT-10 progenitor strain: any decreases in fitness would likely result in non-viable cells. Figure 3f summarizes the overall correlation between the fitness of the donor strain (OT) and the average fitness of RT strains. The results underscore the enormous variability in the fitnesses of the RT strains, and reveal the poor "donor-offspring" correlation of their relative fitnesses. When examined as distinct groups, the distribution of fitness values for OTs ($N = 27$) was very similar to that of RTs ($N = 24$) (Fig. 3g). Here again, the growth rates suggest a possible interplay between selection within the limited array of mutations that may have arisen in the isogenic recipient strain and the transient—but nonetheless functionally critical—transcriptional response of the cell to the chimeric RNAse P RNA it relies upon.

Effects of Complementation on RNA Levels of *rnpB*, *rnpA* and RNase P Substrates

We explored the molecular correlates of heterologous substitution of the *rnpB* gene by measuring the levels of both *rnpA* and *rnpB* RNA in control and experimental lines. We selected three experimental lines among the faster growing strains (OT-18, RT-5-126, RT-10-2, with relative fitnesses of 0.48, 0.49, and 0.37, respectively; see Supplementary Table 5) to test whether any specific changes in the levels of these key transcripts, relative to wild type, were shared among them and correlated with their relative fitness. Quantitative RT-PCR showed no difference in levels of *rnpA* transcripts in two out of three strains tested, and a twofold increase in transcription of *B. subtilis rnpB* compared to the control strain (Supplementary Fig. 5; Supplementary Table 4). While increased transcription of the heterologous *rnpB* gene may be a response to the reduced efficiency of the chimeric RNase P, it failed to restore cell fitness to control strain levels (Supplementary Fig. 5).
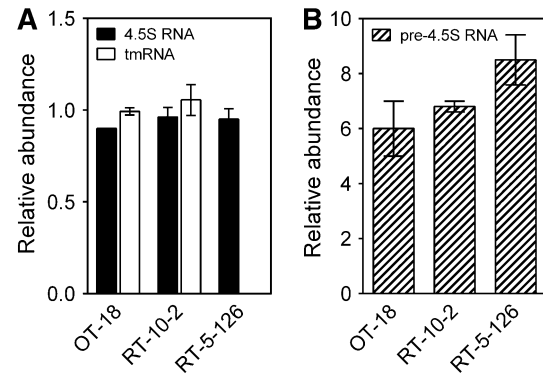

Fig. 4 Comparison of levels of RNase P substrates in DW2/pFLP-Bs relative to the control strain. **a** Levels of total 4.5S RNA and total tmRNA measured in three DW2/pFLP-Bs lineages were comparable to control strain levels. **b** In contrast, the levels of precursor 4.5S RNA transcripts for the same lineages shown in (**a**) were up to ~8.5-fold higher than the control strain. RNA abundance was normalized to levels measured in the control strain. Mean relative abundance was calculated from 2 or 3 independent quantitative PCR assays; data for 4.5S RNA in OT-18 were from 2 independent assays and in each of these, the values for relative levels were identical (0.9), therefore, no error bars are shown. Levels of tmRNA were not assayed in lineage RT-5-126. *Error bars show* $\pm$SD

The multifaceted role played by RNase P—and the many substrates it is capable of processing—led us to investigate the fate of specific RNase P substrates in complemented strains. We assessed the severity of RNase P processing defects in DW2/pFLP-Bs by measuring total and precursor levels of RNase P substrates: 4.5S RNA; tmRNA; and three specific tRNAs, tRNA^{Leu}, tRNA^{Phe} and tRNA^{Lys}. Figure 4a shows the total level 4.5S RNA and tmRNA in the three DW2/pFLP-Bs transformant lines, plotted relative to the levels measured in the control strain: no significant differences can be seen. In contrast, we detected a significant accumulation of 4.5S precursor substrates, ranging from 6 to 8.5-fold relative to the control strain (Fig. 4b). Compared to the control strain, where approximately 96 % of all 4.5S RNA present in the cell is in the processed (mature) form, the DW2/pFLP-Bs lines only exhibit between 65 and 73 % of total 4.5S RNA in processed form (Fig. 5). We interpret this altered ratio as indicating reduced processing by the chimeric RNase P. Interestingly, not all of the RNase P substrates we examined suggest reduced processing capacity for the chimeric RNase P. In two of the three strains tested, only pre-tRNA^{Leu} showed significant accumulation relative to the control strain (Fig. 6). Because the substrates we examined in this study represent but a fraction of the RNA species that are processed by RNAse P, we are not in a position to generalize our results of the consequences of sub-optimal RNAse P processing either for the remaining tRNAs or for other RNA substrates.
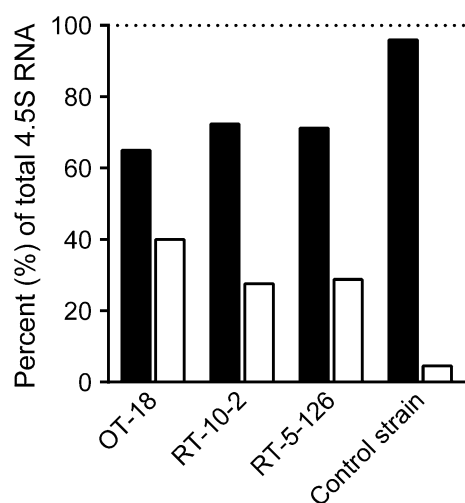
Fig. 5 DW2/pFLP-Bs lineages have lower levels of mature 4.5S RNA compared to the control strain. To assess changes in RNase P substrates available in mature form, using data from a single assay (one among the three assays shown in Fig. 4) we estimated mature (*black bars*) and precursor (*white bars*) 4.5S RNA levels for three DW2/pFLP-Bs lineages and the control strain. Quantitative PCR results were normalized to the total levels found in each strain separately, where *mature = total − precursor*. Estimate levels of mature 4.5S RNA transcripts in DW2/pFLP-Bs represented approximately 75 % or less of the total, compared to 96 % in the control strain. Data are shown as *percent (%)* of total 4.5S RNA (*dotted line*)

Genome-Wide Changes in Complemented Cells

To gain a broader perspective on the consequences of *rnpB* heterologous replacement, we contrasted the global patterns of expression in the control strain and DW2/pFLP-Bs strains. We expected that this analysis might reveal the differential expression of known RNase P substrates, but might also reveal other transcriptional consequences of *rnpB* replacement [previous studies have succeeded in extracting this type of information from genome-wide transcriptome analyses of RNase P *E. coli* mutant strains, see (Lee et al. 2008) for strain KS2003 (*rnpB* mutant) and (Li and Altman 2003; Li et al. 2003) for strain *rnpA49* (*rnpA* mutant)]. We predicted that the DW2/pFLP-Bs mutants undergo global transcriptional changes, including some that could contribute to mitigating the deleterious effects of heterologous replacement.

We characterized the transcriptional differences between the control strain and the DW2/pFLP-Bs strains using microarray analysis (data deposited in the NCBI Gene Omnibus Expression (GEO) Database, GEO series accession #GSE51639, available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51639). The genes that exhibited differential expression [defined here as >1.7-fold increase or decrease, following (Yang et al. 2002)] are summarized in Supplementary Table 3. We consider these changes to be the consequences, both direct and indirect, of
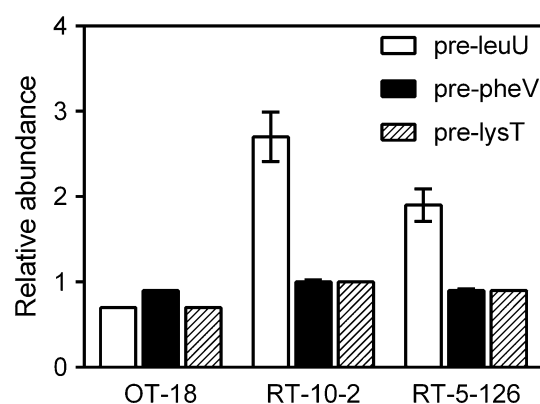

Fig. 6 Changes in levels of precursor tRNAs in DW2/pFLP-Bs relative to the control strain. Levels of transcripts for *pre-leuU* were up to threefold higher in DW2/pFLP-Bs relative to the control strain, whereas levels for *pre-pheV* and *pre-lysT* were comparable to the control strain. Data represent averages calculated from 2 or 3 independent assays where available; *error bars* show ±SD

chimeric RNase P activity in vivo (Waugh and Pace 1990; Wegscheid et al. 2006; Li et al. 2003; Jovanovic et al. 2002; Buck et al. 2005a). We identified 79 genes distributed across a number of COG categories that show reproducible and significant expression changes as the result of the heterologous replacement of the *rnpB* gene (Supplementary Fig. 6). Based on our initial microarray experiments we chose 12 putatively differentially expressed genes to validate by quantitative PCR (qPCR), comparing expression in three independent DW2/pFLP-Bs strains against the expression in a single control DW2/pFLP-Ec strain. Three genes (*glcF*, *rplV* and *inf-c*) that showed no differential expression in our microarray experiments were used as controls. Two of these, *rplV and inf-c*, showed identical expression across all four lines (*glcF* showed identical expression in 3 of the 4 sampled lines). Our qPCR results validate our microarray findings for 8 differentially expressed genes (*hisH*, *leuB*, *pheA*, *stpA*, *tnaA*, *lldD*, *lldP*, *lldR*) in at least two of the experimental lines. The remaining 4 genes (*ilvN*, *rbsD*, *pheS*, *espF*) we investigated showed results inconsistent in either significance or direction with our initial microarray data.

Putative Novel Polycistronic Operon RNase P Substrates

Several genes differentially expressed in DW2/pFLP-Bs were localized to polycistronic operons: 26 of the genes that showed significant changes with respect to the control strain belong to 10 polycistronic operons (Supplementary Table 2). Each of these polycistronic operons contained 2 or more genes with a twofold or greater change (increase or decrease) in abundance, but always in the same direction.

In addition to previously recognized RNase P substrates (e.g., tna and rbs operons) (Li and Altman 2003; Li et al. 2003), we also detect differential expression in the pheST-himA, manXYZ-yobD, nuoA-N, cvpA-purF-ubiX, pheLA, ftsXEY, lldPRD, and treBC operons [naming conventions as in (Keseler et al. 2011)].

Discussion

Our results confirm the ability of the *B. subtilis rnpB* gene to complement a deletion of its homolog in *E. coli* (Waugh and Pace 1990; Wegscheid et al. 2006) despite considerable differences in their sequences and secondary structures (Fig. 1). This replacement, however, clearly exacts a cost: the cell survives, but growth is impaired. Surprisingly, this cost in cell fitness, measured as maximal growth rate, varies substantially among isogenic lines derived from a single transformation experiment. This variability suggests that the perturbation introduced by the impaired performance of the chimeric RNase P while severe, can be mitigated in the recipient cell.

We cannot formally rule out compensatory changes in the chromosome of the host strain as the source of this fitness variability without sequencing the chromosome in its entirety (Sniegowski et al. 1997; Sniegowski and Gerrish 2010), although the isogenic recipient line is unlikely to have accumulated sufficient variability, let alone a wide enough array of compensatory mutations, to fully account for our results. We have, however, ruled out any mutations in the *rnpB* gene itself and in its adjacent control regions. Furthermore, our re-transformation experiments (Fig. 3a–f) suggest that the direction of the fitness change cannot be predicted. Our experiments obviously select for viability, but they do not select for fast growth: at no point are the transformant cells in competition with one another. We conclude that the variability in growth rate cannot be solely attributed to changes in the chromosomal background occurring after transformation, since we observe the same range of relative fitnesses in both OT and RT groups (0.03–0.57 and 0.05–0.65, respectively) (Fig. 3g). Furthermore, whereas compensatory mutations would always have the net effect of increasing fitness, as their name suggests, we observe fitness decreases in the re-transformants as frequently as we see increases. Finally, we note that no compensatory mutations affecting growth rate are seen in our control lines, despite the displacement of the *rnpB* gene away from its chromosomal location and onto our experimental plasmid system.

The reduction in fitness brought about by the heterologous replacement of the *rnpB* gene may be, in part, the result of reduced processing by the chimeric RNase P. We detect no reduction in the expression of *B. subtilis rnpB* RNA, and endogenous *rnpA* mRNA levels were similar to the control strain for two out of three strains tested (Supplementary Fig. 5). When known substrates of RNase P are scrutinized, however, the ratio of precursor to mature 4.5S RNA (Fig. 5) and levels of pre-tRNA^{Leu} (Fig. 6) appear seriously affected, suggesting a bottleneck in the processing of these substrates by the chimeric RNase P. These results are consistent with previous reports detailing RNase P processing disturbance (Bourgaize and Fournier 1987; Dong et al. 1996; Wegscheid and Hartmann 2007; Gossringer et al. 2006).

A broader perspective on the consequences of *rnpB* replacement on genome-wide transcription reveals an unexpectedly large number of affected genes. Some of these include previously identified RNase P substrates (Li and Altman 2003; Li et al. 2003; Mohanty and Kushner 2007): changes in their expression suggest regulatory feedback between the levels of processing and transcription. The list of affected genes, however, includes many unexpected entries, distributed throughout functional classes. While these changes may represent the non-specific consequences of cell stress, their repeatability across experiments leads us to a different conclusion. We propose that these changes reflect an adaptive response on the part of the cell that acts to mitigate the fitness costs of *rnpB* replacement. In this scenario, the emergence and eventual fixation of compensatory mutations are time-dependent processes, since our initial population is genetically homogenous. While awaiting the appearance and eventual fixation of compensatory mutations, however, cells saddled with a suboptimal RNase P still have recourse to a transcriptional response—a response that mitigates the consequences of our experimental perturbation.

The variability in growth rate we observe thus reflects the ability of individual cells to mount this transcriptional response, an ability that may depend on stochastic factors or on small differences in the physiological state of the cell (Martinez-Antonio and Collado-Vides 2003; Smits et al. 2006; Cases and de Lorenzo 2005). Because RNase P activity can control the rate of tRNA availability, as well as that of so many other small RNAs (tmRNAs, 4.5S RNA), inhibiting its performance can have deep and wide-ranging effects. Conversely, however, the connectivity of RNase P may provide the cell with a number of possible transcriptional responses that dampen the perturbation.

Seen in this light, transcription acts as a short-term mechanism of adaptation that bridges the gap between the perturbation and the appearance or fixation of one or more compensatory mutations. This connection between the physiological dynamics of the transcriptome (short-term) and the evolutionary dynamics of the genome (long-term) deserves further scrutiny (Ostrowski et al. 2008; Travisano and Lenski 1996; Barrick et al. 2010; Clune et al. 2008).

Alleviating the deleterious effects of *rnpB* complementation involves alternative processes yet to be fully characterized. These processes include compensatory mutations, modification of the transcription rates of RNase P substrates, as well as higher order compensatory mechanisms. Our study also expands on previous gene expression reports for RNase P mutants and sheds light on the multiple metabolic processes that depend, in whole or in part, on RNase P function.

By assessing the fitness costs involved in *rnpB* exchange, we are probing the actual extent of functional equivalence between Type-A and Type-B RNase P RNAs in further detail. As a ribozyme, bacterial RNase P RNA can cleave substrates in vitro, in the absence of its protein subunit, but only under conditions of high ionic strength (Guerrier-Takada et al. 1983). In vitro, the rnpA protein of *E. coli*, but not that of *B. subtilis*, stabilizes the overall tertiary structure of the RNase P holoenzyme into its functional folded form (Buck et al. 2005a). It is possible that in our mutants, the *E. coli* rnpA protein retained its stabilizing role despite being paired with *B. subtilis* RNase P RNA, mitigating the fitness consequences of the replacement. The rnpA protein has also been shown to produce changes in the conformation of bases near the tRNA-binding site of its cognate RNase P RNA (Buck et al. 2005b). If an incompatibility between the *B. subtilis* *rnpB* RNA and the *E. coli* rnpA protein disrupts or abolishes this conformational change, the efficiency with which the holoenzyme recognizes the 5′ leader sequences of incoming substrates will likely be affected.

This study confirms that the RNA component of *E. coli*'s RNase P can be successfully replaced with a highly divergent heterolog that shares little sequence similarity (Chen et al. 1998; Harris et al. 2001). This result is all the more surprising given the extent to which the RNA component of RNase P determines important functional characteristics of the ribonucleoprotein including its relative preference for precursor versus mature tRNAs (Buck et al. 2005a). The capacity to tolerate divergence while still maintaining functional interchangeability may be particularly pronounced for catalytic RNAs associated (as most are) with one or more protein cofactors, and may hint at the convergent evolution of these ribozyme catalysts. The interchangeability and fitness effects of replacing the *E. coli* rnpA protein have been described in detail (Turrini et al. 2012), and an extreme example of the interchangeability of eukaryotic RNase Ps has recently been described by Weber et al. (2014).

Our results also hint at a general system-level mechanism underlying the extensive horizontal gene exchange seen in Bacteria (Syvanen 2012). While the incorporation of heterologous genetic information into a genome in

which it has not coevolved will most often lead to decreased fitness, a transcriptional response such as the one we describe may dampen this effect. This, in turn, may provide recipient lineages with the time required for more permanent compensatory mutations to emerge and fix, leading to an overall increase in the frequency of evolutionarily successful horizontal transfer events.

Methods

The following methods are described in detail in Supporting Information. Cells were routinely grown in nutrient rich media supplemented with appropriate antibiotic. pFLP plasmids contained a kanamycin resistance marker and a FLP cassette and *rnpB* transcription in both pFLP-Ec and pFLP-Bs was controlled by native *E. coli* promoter (Lee et al. 1989) and terminator sequences (Supplementary Fig. 2b). pRescue contained a gentamycin resistance marker and two FRT sites oriented in the same direction, allowing the plasmid to be linearized in the presence of FLP recombinase (Supplementary Figs. 1c, d, 2a). Transformations were performed with pFLP-Bs and in parallel, pFLP-Ec as positive control (Supplementary Fig. 1b). Transformants were confirmed by antibiotic profiling, diagnostic digest of plasmids and with quantitative PCR targeting both *E. coli* and *B. subtilis* *rnpB* transcripts. Relative levels of gene expression were calculated as previously described (Livak and Schmittgen 2001) and were normalized to levels in the control strain. Graphical Analyzer v.3 software (Vernier Software and Technology, Beaverton, OR) was used to calculate the maximal slope for the exponential growth phase of each strain; the maximal slope of the control strain was normalized to 1. The relative fitness of each DW2/pFLP-Bs transformant was calculated by comparing its maximal slope to the normalized control strain slope specific to each experiment. Average relative fitnesses are reported for lineages that were assayed for growth in two or more experiments. Microarrays were performed using the 3DNA Array 900MPX Expression Array Detection Kit (Genisphere). Slides were scanned with GenePix Pro v.4.1 software (Axon Instruments, Inc.). Any gene feature with a signal-to-background ratio of 2 or greater was excluded from analyses. Differential expression was defined as a 1.7-fold increase or decrease in expression relative to wild type. This cut-off was chosen based on methods by (Yang et al. 2002); we performed self–self hybridizations of DW2/pFLP-Ec and only 5 % of data points were detected beyond this limit. The ratios of median intensity were calculated as wild type/mutant, which in our case was DW2/pFLP-Ec over DW2/pFLP-Bs.

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