Study of the functionality of the *Helicobacter pylori* trans-translation components SmpB and SsrA in an heterologous system

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**Abstract**

**Background:** Trans-translation is a ubiquitous bacterial quality control-mechanism for both transcription and translation. With its two major partners, SsrA a small stable RNA and the SmpB protein, it promotes the release of ribosomes stalled on defective mRNAs and directs the corresponding truncated proteins to degradation pathways. We have recently shown that trans-translation is an essential function in the gastric pathogen *Helicobacter pylori*. Our results suggested that some properties of the *H. pylori* trans-translation machinery distinguishes it from the well known system in *E. coli*. Therefore, we decided to test the functionality of the SmpB and SsrA molecules of *H. pylori* in the *E. coli* heterologous system using two established phenotypic tests.

**Results:** *H. pylori* SmpB protein was found to successfully restore the *E. coli ΔsmpB* mutant growth defect and its capacity to propagate λimmP22 phage. We showed that in *E. coli*, *H. pylori* SsrA (Hp-SsrA) was stably expressed and maturated and that this molecule could restore wild type growth to the *E. coli ΔssrA* mutant. Hp-SsrA mutants affected in the ribosome rescue function were not able to restore normal growth to *E. coli ΔssrA* supporting a major role of ribosome rescue in this phenotype. Surprisingly, Hp-SsrA did not restore the phage λimmP22 propagation capacity to the *E. coli ΔssrA* mutant.

**Conclusions:** These data suggest an additional role of the tag sequence that presents specific features in Hp-SsrA. Our interpretation is that a secondary role of protein tagging in phage propagation is revealed by heterologous complementation because ribosome rescue is less efficient. In conclusion, tmRNAs present in all eubacteria, have coevolved with the translational machinery of their host and possess specific determinants that can be revealed by heterologous complementation studies.

**Background**

Trans-translation is a quality-control mechanism that is ubiquitous in bacteria and involves two activities [1-3]. First, trans-translation favors the rescue of ribosomes stalled on defective or damaged mRNAs (lacking a stop codon) through the restart of translation. Second, trans-translation functions to direct incomplete peptides to degradation by the addition of a specific tag [4]. Trans-translation is generally non-essential and requires two factors: SsrA, a small stable structured RNA (also called tmRNA) that acts both as a tRNA by its alanylated tRNA-like domain (TLD) and as a mRNA-like domain (MLD) [4] and its protein cofactor, SmpB.

The length and sequence of the trans-translation appended peptide tag varies with the bacterial species (between 8 and 35 amino acids) [5]. Mostly studied in *E. coli*, the tag encoded by SsrA is sufficiently informative to target any trans-translated proteins to degradation pathways [4]. The phenotypes of mutants deficient in this process depend on the species examined and are related to environmental adaptation, differentiation, stress response or virulence (for a review see [6]). Growing evidence indicates that trans-translation tagging targets specific substrates and therefore plays a regulatory role in organisms such as *Caulobacter crescentus* [7,8] *Yersinia pseudotuberculosis* [9], *Helicobacter pylori* [10] or *Streptomyces coelicolor* [11].
In *E. coli*, numerous phenotypes were associated with the deficiency of *trans*-translation, among which a slight enhancement of the doubling time that was observed even under normal growth conditions [12]. One of the tools used to characterize the SsrA determinants *in vivo* was the dependence on *trans*-translation of the growth of the hybrid bacteriophage \( \lambda imm^R \) in *E. coli* [13–15]. This phage is a hybrid between the *E. coli* lambda phage and the *Salmonella* P22 phage and is specific for *E. coli*. *E. coli* strains defective in *trans*-translation display a characteristic phenotype termed "Sip" (for selectively inhibited) [13]. Indeed, the frequency of infection by \( \lambda imm^R \) is 10,000-fold lower in \( \Delta smpB \) or \( \Delta ssrA \) *E. coli* mutants as compared to that in the corresponding parental strain [13,16]. The precise molecular basis of the phage plating defect in *trans*-translation-deficient cells is not yet understood. The impact of SsrA point mutations on \( \lambda imm^R \) growth in *E. coli* was first analyzed by Withey and Friedman [14] who showed (i) that charging of tmRNA with Ala was essential and, (ii) that degradation of proteins tagged by tmRNA was only required to achieve optimal levels of phage growth. A more recent study challenged these conclusions and demonstrated that \( \lambda imm^R \) propagation in *E. coli* is exclusively dependent on ribosome recycling functions of *trans*-translation and not on its proteolysis targeting activity [15].

We have recently investigated the role of *trans*-translation in *Helicobacter pylori* [10]. *H. pylori* is a bacterial pathogen that colonizes the stomach of half of the human population and is strongly adapted to persist and multiply under stressful conditions such as low pH. Colonization of the stomach by *H. pylori* is associated with several gastric pathologies ranging from gastritis, peptic ulcer to adenocarcinoma [17]. We demonstrated that ribosome rescue by *trans*-translation is essential for *in vitro* growth of *H. pylori*. Interestingly, stress resistance and natural competence were strongly affected in *H. pylori* strains carrying a mutated tmRNA tag sequence [10]. While the overall structure of *H. pylori* SsrA is conserved, the tag sequence significantly differed from that of *E. coli* and our mutagenesis study revealed both identical and different properties as compared to its *E. coli* homolog [10]. To investigate further these differences using a model organism, we decided to study the *H. pylori* SmpB and SsrA expressed in the *E. coli* heterologous system.

**Results**

**Functional complementation of an *E. coli* smpB deletion mutant by Hp-SmpB**

To examine the functionality of the SmpB protein of *H. pylori* (Hp-SmpB) in *E. coli*, the corresponding gene *hp1444* was amplified from *H. pylori* strain 26695 and cloned into pILL2150 under control of an inducible promoter, to generate pILL786 (Table 1). This plasmid was transformed into *E. coli* wild type strain MG1655 and its isogenic \( \Delta smpB \) mutant [18] (Table 1 and 2). Expression of Hp-SmpB in *E. coli* was verified by western blot in the \( \Delta smpB \) mutant using antibodies raised against purified *E. coli* SmpB. Hp-SmpB was detected, its synthesis was strongly enhanced upon addition of IPTG and was overexpressed in comparison with the *E. coli* endogenous SmpB protein, Ec-SmpB (Figure 1).

The efficacy of propagation of the hybrid phage \( \lambda imm^R \) [13] was measured on different strains. Table 3 presents the relative efficiency of plating (EOP) of each strain in comparison with that of the wild type parental strain. Phage propagation on strain MG1655 \( \Delta smpB \) containing the empty vector pILL2150 was, as expected, strongly affected with an EOP of \( 1.3 \times 10^{-5} \) (Table 3). Relative EOP of strain MG1655 \( \Delta smpB \) pILL786 in the presence of IPTG, expressing Hp-SmpB is close to 1 (Table 3). This result demonstrated that Hp-SmpB is active in *E. coli* and efficiently complemented the phage propagation defect phenotype. In addition, the growth defect of MG1655 \( \Delta smpB \) mutant was analyzed with or without Hp-SmpB. Under our test conditions, MG1655 \( \Delta smpB \) mutant presented a doubling time that was about twice that of the wild type strain and was restored to wild type growth in the presence of Hp-SmpB expressed by pILL786 (Figure 2 and Table 3). This indicated that Hp-SmpB is able to replace Ec-SmpB functions during *trans*-translation in *E. coli*.

**Expression and maturation of Hp-SsrA in *E. coli***

To evaluate the heterologous complementation capacity of Hp-SsrA in *E. coli*, we constructed pILL788 and pILL2318 carrying the ssrA gene of *H. pylori* under control of a promoter on high copy and low copy number plasmids, respectively (Table 1). Plasmids pILL788 and pILL2318 expressing wild type Hp-SsrA were trans-

![Figure 1 Detection of SmpB in *E. coli*. Detection of SmpB protein in *E. coli* was performed by western blot with an *E. coli* SmpB polyclonal antibody. Lane 1: wild type *E. coli* strain (predicted MW SmpB\(^{Ec}\) = 18,125 Da), lane 2: \( \Delta smpB \) *E. coli* mutant. Lanes 3-4: SmpB\(^{Ec}\) detection in a \( \Delta smpB \) *E. coli* mutant carrying the inducible vector pILL786 expressing the smpB\(^{Ec}\) gene (predicted MW SmpB\(^{Ec}\) = 17,682 Da), with or without induction with 1 mM IPTG, respectively. Calibrated amounts of crude bacterial extracts were separated by SDS-15% PAGE. MW: molecular weight.]
formed into both MG1655 wild type and ΔssrA strains (Table 2). The expression of Hp-SsrA was examined by northern blot with total RNA extracted from different E. coli strains and from the H. pylori 26695 strain (Figure 3). A 300 nt long riboprobe was chosen in the region of Hp-SsrA displaying homology with Ec-SsrA. A band of 386 nt that matches the size of the mature Hp-SsrA was detected in the RNA samples extracted from E. coli MG1655 ΔssrA pILL788 and MG1655 ΔssrA pILL2150 strains (Figure 3). As expected, the amount of Hp-SsrA is weaker when expressed from the low copy plasmid pILL2318 than from pILL788. With RNA extracted from H. pylori strain 26695, we observed an intense band of the same size that was absent in samples extracted from MG1655 ΔssrA containing pILL2150, the empty vector (Figure 3). A faint band corresponding to mature Ec-SsrA (363 nt) was detected in E. coli MG1655 wild type strain. This indicates that in E. coli, Hp-SsrA is expressed and correctly maturated.

### Analysis of the functionality of Hp-SsrA in E. coli

The capacity of Hp-SsrA to complement the phage propagation defect of an E. coli strain deficient in SsrA was examined. The EOP of strain MG1655 ΔssrA pILL2150 (empty vector) was 2.6 × 10⁻⁵ as expected (Table 3). Surprisingly, the presence of pILL788 expressing processed Hp-SsrA in strain MG1655 ΔssrA did not restore the capacity to propagate phage λimmP22 (Table 3). This showed that Hp-SsrA is not able to replace Ec-SsrA in this phenotypic test. It was controlled that phage λimmP22 propagation was restored in strain MG1655 ΔssrA pILL2334 expressing wild type Ec-SsrA. Under our test conditions, the doubling time of E. coli ΔssrA mutant was twice that of the wild type strain (Figure 2). Interestingly, wild type growth was restored in the E. coli ΔssrA mutant complemented with plasmid pILL788 that expresses high levels of Hp-SsrA (Figure 2) but not with plasmid pILL2318 that expresses low levels of Hp-SsrA. As a control, wild type growth was also observed with strain MG1655 ΔssrA pILL2334 expressing wild type Ec-SsrA. This indicated that Hp-SsrA is functional to rescue the growth defect of E. coli ΔssrA but is not able to restore the phage propagation deficiency. We then wanted to understand further the functional basis of the partial functionality of Hp-SsrA in E. coli.

### Table 1: Plasmids used in this study

| Plasmids       | Relevant features                                      | Reference  |
|----------------|-------------------------------------------------------|------------|
| pEXT21         | low copy number E. coli vector                        | [25]       |
| pILL2318       | H. pylori ssrA⁷ cloned into pEXT21                    | This study |
| pILL2150       | high copy number H. pylori/E. coli shuttle vector      | [24]       |
| pILL22334      | E. coli ssrA⁷ cloned into pILL2150                    | This study |
| pILL786        | hp1444 encoding Hp-SmpB cloned into pILL2150         | This study |
| pILL788        | H. pylori ssrA⁷ cloned into pILL2150                  | [10]       |
| pILL791        | H. pylori ssrA⁰⁰ cloned into pILL2150                 | [10]       |
| pILL792        | H. pylori ssrA⁹⁹ cloned into pILL2150                 | [10]       |
| pILL793        | H. pylori ssrA⁹⁹ cloned into pILL2150                 | [10]       |
| pILL794        | H. pylori ssrA⁹⁹ cloned into pILL2150                 | [10]       |
| pILL2328       | H. pylori ssrA⁹⁹ cloned into pILL2150                 | [10]       |
Analysis of the functionality of mutated Hp-SsrA versions in E. coli

In a previous study, we constructed a series of five H. pylori SsrA mutants and evaluated in H. pylori their impact on trans-translation, survival and stress-response [10]. Characteristics of these mutations are summarized in Figure 4. Plasmids pILL793, pILL794 and pILL792 express mutant Hp-SsrA that are unable to be alanylated on the TLD (SsrAwobble), to interact with SmpB (SsrASmpB) and to restart the translation on the MLD (SsrAresume), respectively. Each of this mutation was found to be essential for growth of H. pylori [10]. When these plasmids were tested for complementation of the E. coli ΔssrA mutant, neither phage propagation nor growth defective phenotypes was rescued (Figure 2 and Table 3).

In H. pylori, two mutations in the MLD of Hp-SsrA were found to be viable but affected the capacity of the corresponding mutant strains to resist to various stresses [10]. One mutation targets the terminal part of the tag sequence, the corresponding mutant gene Hp-SsrA30 is

### Table 2: E. coli strain used in this study.

| Strains                  | ssrA and smpB alleles                                      | Plasmids [antibiotic resistance] |
|--------------------------|------------------------------------------------------------|----------------------------------|
| MG1655 pILL2150          | smpBEc ssrAEc/pILL2150                                     | multicopy [Cm]                   |
| MG1655 pEXT21            | smpBEc ssrAEc/pEXT21>                                      | low copy [Sp]                    |
| MG1655 ΔsmpB pILL2150    | ΔsmpBEc ssrAEc/pILL2150                                    | multicopy [Cm]                   |
| MG1655 ΔsmpB pILL786     | ΔsmpBEc ssrAEc/pILL2150 with smpBe                                   | multicopy [Cm]                   |
| MG1655 ΔssrA pILL2150    | smpBEc ΔssrAEc/pILL2150 with smpBEc                               | multicopy [Cm]                   |
| MG1655 ΔssrA pILL2334    | smpBEc ΔssrAEc/pILL2334 with ssrAEc-WT                        | multicopy [Cm]                   |
| MG1655 ΔssrA pILL788     | smpBEc ΔssrAEc/pILL2150 with smpBEc-WT                        | multicopy [Cm]                   |
| MG1655 ΔssrA pILL2318    | smpBEc ΔssrAEc/pEXT21 with ssrAHp-WT                         | low copy [Sp]                    |
| MG1655 ΔssrA pILL791     | smpBEc ΔssrAEc/pILL2150 with ssrAHp-DD                       | multicopy [Cm]                   |
| MG1655 ΔssrA pILL792     | smpBEc ΔssrAEc/pILL2150 with ssrAHp-resume                   | multicopy [Cm]                   |
| MG1655 ΔssrA pILL793     | smpBEc ΔssrAEc/pILL2150 with ssrAHp-wobble                   | multicopy [Cm]                   |
| MG1655 ΔssrA pILL794     | smpBEc ΔssrAEc/pILL2150 with ssrAHp-smpB                     | multicopy [Cm]                   |
| MG1655 ΔssrA pILL2328    | smpBEc ΔssrAEc/pILL2150 with ssrAHp-STOP                     | multicopy [Cm]                   |

The doubling time of each E. coli strain was calculated from growth curves performed in LB medium at 37°C with chloramphenicol [Cm] 100 μg/ml or with spectinomycin [Sp] 100 μg/ml.
carried by plasmid pILL791. This mutation was chosen because it was described to stabilize the trans-translated proteins in species like *E. coli*. In another mutant, Hp-SsrASTOP (carried by pILL2328) two stop codons were introduced immediately downstream from the resume codon. As a consequence, Hp-SsrA STOP adds a minimal tag (Ala-Val) to trans-translated proteins (Figure 4).

These two mutated Hp-SsrA versions did not restore the phage propagation capacity to the *E. coli* ΔssrA mutant (Table 3). Interestingly, growth defect of the *E. coli* ΔssrA mutant was restored to the wild type level by complementation with pILL791 expressing Hp-SsrA DD, and not with pILL2328 expressing Hp-SsrA STOP.

**Discussion**

Trans-translation is a bacterial ubiquitous mechanism of quality-control for protein and mRNA synthesis. We have recently shown that trans-translation is essential for *in vitro* growth of the gastric pathogen *H. pylori* [10] like in a few other human pathogens, *Mycoplasma genitalium* [19], *Neisseria gonorrhoeae* [20] or *Haemophilus influenzae* [21]. We also demonstrated that in *H. pylori*, the essential trans-translation function is ribosome rescue and that a single ribosomal translocation step is sufficient to promote release of stalled ribosomes [10]. Using different mutants of *H. pylori* ssrA, we found that under conditions of functional ribosome rescue, the tagging of trans-translated proteins was required for tolerance to both oxidative and antibiotic stresses and for effective natural competence. These data revealed for the first time that control of protein degradation through trans-translation is by itself central in the management of stress conditions and of competence and supports a regulatory role of trans-translation dependent protein tagging. Since we anticipate that this regulatory role of protein tagging is underestimated in *E. coli* and because we possessed a collection of well-defined Hp-SsrA mutants, we decided to explore the functionality of the *H. pylori* trans- translational components in *E. coli*.

Measurement of the λimmP22 phage propagation is a classical test to evaluate the functionality of trans-translation in *E. coli*. As previously reported, both ΔssrA and ΔsmpB *E. coli* mutants exhibit a 10,000-fold defect of phage propagation [14]. *E. coli* SsrA mutants present a slight growth defect, enhanced sensitivity to stress and to sub-inhibitory antibiotic concentrations. These phenotypes are complemented by *E. coli* SsrA variants that add a tag lacking some proteolytic determinants (f.i SsrA DD). Therefore, these phenotypes are likely not to depend on proteolysis.
In a first test, *H. pylori* SmpB protein was found to successfully complement the *E. coli* ΔsmpB mutant for both phage propagation and growth despite only 34.6% identity between Ec-SmpB and Hp-SmpB. This showed that Hp-SmpB is able to interact with both the *E. coli* SsrA RNA and ribosomes to perform efficient trans-translation in *E. coli*.

Results with Hp-ssrA in *E. coli* revealed a more complex picture. First, we showed that upon expression in *E. coli*, Hp-SsrA is highly expressed and exhibits a size compatible with correct maturation. Indeed, Hp-SsrA and Hp-SsrA WT restored a wild-type growth phenotype to an *E. coli* ΔssrA mutant indicating its functionality in *E. coli*. This result is in agreement with a minor role of the protein tagging step in the growth defect of *Ecoli* ΔssrA. Accordingly, we observed that the mutant versions of Hp-SsrA that were affected in ribosome rescue (SsrA Resume, SsrA wobble and SsrASmpB) failed to complement the slow growth phenotype of *E. coli* ΔssrA. Unexpectedly, the Hp-SsrA STOP mutant that contains an intact resume codon followed by two stop codons is not able to complement the *E. coli* ΔssrA growth defect. This is surprising since in *H. pylori*, the SsrA STOP mutation is not essential for in vitro growth strongly suggesting that it is still effective in

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**Table 3: Ability of *H. pylori* SmpB and of wild type or mutant alleles of ssrAHp to support growth of λimmP22 in *E. coli* ΔssrA or ΔsmpB deletion mutants and to restore the growth defect in *E. coli* ΔssrA or ΔsmpB mutants**

| Strains        | ssrA or smpB alleles       | EOP§      | Growth defect restoration in *E. coli* ΔsmpB or in *E. coli* ΔssrA |
|----------------|-----------------------------|-----------|---------------------------------------------------------------|
| MG1655         | smpB Ec ssrA Ec             | 1         | -                                                            |
| MG1655 ΔsmpB   | smpB Ec ssrA Ec             | 1.3 × 10^-5 | no                                                          |
| pilL2150       | smpB Ec ssrA Ec/smpB Hp     | 0.6       | yes                                                          |
| MG1655 ΔssrA   | smpB Ec ΔssrA Ec            | 2.6 × 10^-5 | no                                                          |
| pilL2334       | smpB Ec ΔssrA Ec            | 1         | yes                                                          |
| MG1655 ΔssrA   | smpB Ec ssrA Hp-WT          | 5.0 × 10^-5 | yes                                                          |
| pilL788        | smpB Ec ssrA Hp-DD          | 1.6 × 10^-5 | yes                                                          |
| MG1655 ΔssrA   | smpB Ec ssrA Hp-STOP        | 6.1 × 10^-5 | no                                                            |
| pilL2328       | smpB Ec ssrA Hp-resume      | 3.9 × 10^-5 | no                                                            |
| MG1655 ΔssrA   | smpB Ec ssrA Hp-wobble      | 2.3 × 10^-5 | no                                                            |
| pilL792        | smpB Ec ssrA Hp-smpB        | 3.6 × 10^-5 | No                                                            |
| MG1655 ΔssrA   | smpB Ec ΔssrA Ec            | 3.6 × 10^-5 | No                                                            |
| pilL794        | smpB Ec ΔssrA Ec            | 3.6 × 10^-5 | No                                                            |

§ EOP is the ratio of the titer of phage on a lawn of bacteria mentioned in the table divided by the titer of phage on a wild type bacterial lawn.
Figure 3 Detection of SsrA<sub>Hp</sub> expressed in <i>H. pylori</i> and from plasmids in <i>E. coli</i>. A SsrA<sub>Hp</sub> riboprobe was used to perform northern blots and detect the SsrA<sub>Hp</sub> molecule in <i>H. pylori</i> and in <i>E. coli</i> wild type or ΔssrA mutant strains. Pre-SsrA<sub>Hp</sub> indicates a band with the size of non-maturated precursor of SsrA<sub>Hp</sub>. A faint band marked by a star corresponds to cross-hybridization with the SsrA<sub>Ec</sub> that is, as expected, absent in the <i>E. coli</i> ΔssrA mutant.
release of stalled ribosomes [10]. In a previous study [15], an equivalent mutation was introduced into *E. coli* SsrA, however only phage propagation phenotype is reported and no mention was made of the growth rate of this mutant. The most straightforward interpretation of our data is that *trans*-translation by Hp-SsrASTOP in *E. coli* is not efficiently using the resume codon. Indeed, there are striking differences between Hp-SsrA and Ec-SsrA. In particular, the resume codon of Hp-SsrA is GUA encoding Valine and in *E. coli*, the resume codon GCA encodes Alanine (Figure 4) [5]. Replacement of the Ec-SsrA resume codon by GUA or GUC encoding Valine is functional in *E. coli* [22]. However, mass spectrometry analysis revealed that breakage of the peptide tag occurred frequently after certain residues like a Valine encoded by GUA and that these SsrA-tag added to proteins are ineffective in growth competition with ΔssrA mutants [22]. Therefore, we hypothesize that the GUA resume codon of Hp-SsrA is a poor resume codon for *trans*-translation in *E. coli* and that additional downstream sequence compensate for this deficiency. As a consequence, the introduction of two stops immediately after the resume codon as in the Hp-SsrA*STOP* mutant might render this compensation impossible and translation restart ineffective.

These data emphasize the strict constraints on SsrA sequence to achieve ribosome rescue in a given organism. The functionality of Hp-SsrA in *E. coli* was also examined using the phage λimmP22 propagation test. Several studies illustrated in Table 4 conclude that λimmP22 propagation in *E. coli* is mainly dependent on efficient ribosome rescue and that the inactivation of the tagging activity did not affect phage growth. It was also reported that the threshold SsrA function required for plaque formation in *E. coli* is fairly low [23]. Thus, the absence of phage λimmP22 propagation in the *E. coli* ΔssrA expressing wild type Hp-SsrA (that complements growth defect)
was unexpected (Table 3). In contrast to Hp-SsrA, wild-type SsrA from *Neisseria gonorrhoeae* (NG-SsrA) restores phage propagation in *E. coli ΔssrA* [20]. Interestingly, NG-SsrA mutant versions carrying mutations affecting either the ribosome rescue function (NG-SsrAUG) or the functionality of the tag sequence (SsrAOD and SsrAOcher) were defective in complementing the phage propagation in *E. coli ΔssrA*. This suggests that under conditions of heterologous complementation of *E. coli ΔssrA* either with Hp-SsrA (this work) or with NG-SsrA [20], λimmP22 phage propagation requires trans-translation-dependent protein tagging in addition to ribosome rescue. The proposition of a secondary role of protein tagging in λimmP22 propagation in *E. coli* is compatible with the observation by Withey and Friedman [14] that smaller plaques were generated in an *E. coli* strain expressing a SsrA0 mutant that encodes a truncated tag. They postulate that the tag is not necessary for phage propagation but is required to allow an optimal growth of phages.

**Conclusions**

To conclude, heterologous complementation showed that the wild type Hp-SsrA is able to restore normal growth to an *E. coli ΔssrA* mutant suggesting that despite the sequence differences between these molecules, Hp-SsrA acts as a partially functional but not optimal tmRNA in *E. coli*. The tag sequence of Hp-SsrA presents several differences with that of the other studied bacteria, in particular a different resume codon, a charged residue at the end of the tag (Lysine instead of Leucine or Valine) (Figure 4) and the absence of a SspB protein recognition motif. We propose that these differences might account for the inability of the Hp-SsrA to support phage propagation in an *E. coli ΔssrA* mutant. This attributes an additional role of trans-translation dependent tagging for efficient λimmP22 phage propagation in *E. coli*. Our interpretation is that this secondary role of protein tagging is revealed by heterologous complementation because ribosome rescue is less efficient. This emphasizes once again the regula-

| E. coli SsrA version | Effects on SsrA | SsrA tag appended to truncated proteins | EOP§ | Reference |
|----------------------|-----------------|----------------------------------------|------|-----------|
| SsrA<sup>WT</sup>    | Wild type       | ANDENYALAA                             | 1    | [14,15]   |
| SsrA<sub>resume</sub> | Substitution of the resume codon by a stop codon | None | 1.3 × 10<sup>-5</sup> | [14] |
| SsrA<sub>wobble</sub> | Absence of alanylation of the tRNA-like domain of SsrA | None | 5 × 10<sup>-5</sup> | [28] |
| SsrA<sub>SmpB</sub>  | Absence of interaction between SsrA and SmpB | None | N.D. |           |
| SsrA<sup>ODD</sup>   | Substitution of the last two alanine residues of the tag by two aspartate residues | ANDENYALDD | 0.5 – 0.1 | [28] |
| SsrA<sub>STOP</sub>  | Two stop codons added after the resume codon | Minimal tag added | 0.9 | [14] |

§ EOP is the ratio between the titer of phage on a lawn of bacteria expressing one of the indicated SsrA versions and the titer of phage on a wild type bacterial lawn; N.D.: Not determined.
tory role of trans-translation in addition to its quality control function.

In conclusion, tmRNAs found in all eubacteria, have coevolved with the translational machinery of their host and possess specific determinants that were revealed by this heterologous complementation study.

**Methods**

**Bacterial strains and growth conditions**

*Escherichia coli* strain MG1655, MG1655 ΔssrA [18] and MG1655 ΔsmpB [18] were grown at 37°C on solid or liquid LB medium. These strains were used as recipients for plasmids carrying different *H. pylori* genes: *smpB*, *ssrA* and mutant versions of *ssrA* as well as the *E. coli* *ssrA* gene (Table 2). Both antibiotics chloramphenicol (Cm) and spectinomycin (Sp) were used at 100 μg ml⁻¹ and isopro- pyl-β-D-thiogalactoside (IPTG) at 1 mM. *H. pylori* strain 26695 was grown under standard conditions, and harvested in mid-log phase as described in [10]. Doubling times (g values) correspond to the mean generation time.

**Molecular techniques and sequencing**

Plasmids pILL788, pILL791, pILL792, pILL793, pILL794, pILL795, pILL2328 correspond to the *H. pylori* *ssrA*²⁷⁻, *ssrA*²⁷⁻, *ssrA*³⁴⁻, *ssrA*⁸⁻, *ssrA*⁸⁻, *ssrA*⁸⁻ genes cloned into the *E. coli*/*H. pylori* shuttle vector pILL2150 [24], respectively. SsrA mutagenesis has been described in [10]. The *H. pylori* *ssrA* gene amplified by PCR with primers H367 (5'-GGACTAGTAGGAAGAGAATT-AG-TATGC-3') and H368 (5'-GGGTACCTTATCCTTTAAAGTGGTGTTT-CTTTAATCGAATAAAAATCAGG-3') was cloned into pILL2150 using the pEXT21 low copy number vector (1-3 copies per cell) [18] and possesses specific determinants that were revealed by this heterologous complementation study.

**Test of λimmP22 propagation in E. coli**

The efficiency of plating (EOP) strains was determined by plating tenfold serial dilution of phage λimmP22 on top agar mixed with 100 μl *E. coli* overnight liquid culture in LB with 0.4% maltose and 10 mM MgSO₄. The number of CFU ml⁻¹ was calculated for each *E. coli* strain. The EOP is the ratio between the titer of phage on a bacterial lawn of the indicated strain (Table 3) and that of the wild type strain.

**Western blot**

Western blot to detect SmpB proteins was performed with *E. coli* whole cell sonicates prepared as in [26]. Protein concentrations were measured with Bradford assay (Bio-Rad). Twenty μg of crude extracts were separated by 15% SDS-PAGE and blotted on a polyvinylidene difluoride membrane (PVDF, Millipore). Hp-SmpB and Ec-SmpB were detected with rabbit polyclonal antibody raised against Ec-SmpB (a generous gift of B. Felden). Binding of the IgG anti-rabbit coupled peroxidase antibody (Amersham) was revealed with the ECL Plus reagent (Pierce).

**RNA extraction, riboprobe synthesis and northern blot**

RNAs were extracted using the phenol-chloroform method as described in [27]. An *E. coli* 5S rRNA riboprobe was synthesized using both primers H357 (5'-GGGGTAGGCCAGTAGCG CG GTGG-3') and H358 (5'-CTAAATACGGACTACTATAGGCCAGTGCG GTTCCCTACTCTGC-3'). Riboprobes synthesis for *H. pylori* SsrA was as in [10]. The ladder used corresponds to pBR322 vector digested byMspI and labeled at the 5'end with γ³²P ATP. Intensities of the bands were determined with Quantity One Software (Bio-Rad). The northern blot procedure was as described in [10].

**Authors’ contributions**

Conceived and designed the experiments: MT, HDR. Performed the experiments: MT, SA, CE. Analyzed the data: MT, HDR. Wrote the paper: MT, HDR. All authors read and approved the final manuscript.

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