The A-site Finger in 23 S rRNA Acts as a Functional Attenuator for Translocation*

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Taeko Komoda†§, Neusa S. Sato†l, Steven S. Phelps*, Naoki Namba‡, Simpson Joseph¶, and Tsutomu Suzuki‡§2

From the †Department of Chemistry and Biotechnology, Graduate School of Engineering, and §Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656 Japan and the ‡Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92093-0314

Helix 38 (H38) in 23 S rRNA, which is known as the “A-site finger (ASF),” is located in the intersubunit space of the ribosomal 50 S subunit and, together with protein S13 in the 30 S subunit, it forms bridge B1a. It is known that throughout the decoding process, ASF interacts directly with the A-site tRNA. Bridge B1a becomes disrupted by the ratchet-like rotation of the 30 S subunit relative to the 50 S subunit. This occurs in association with elongation factor G (EF-G)-catalyzed translocation. To further characterize the functional role(s) of ASF, variants of Escherichia coli ribosomes with a shortened ASF were constructed. The E. coli strain bearing such ASF-shortened ribosomes had a normal growth rate but enhanced +1 frameshift activity. ASF-shortened ribosomes showed normal subunit association but higher activity in poly(U)-dependent polyphenylalanine synthesis than the wild type (WT) ribosome at limited EF-G concentrations. In contrast, other ribosome variants with shortened bridge-forming helices 34 and 68 showed weak subunit association and less efficient translational activity than the WT ribosome. Thus, the higher translational activity of ASF-shortened ribosomes is caused by the disruption of bridge B1a and is not due to weakened subunit association. Single round translocation analyses clearly demonstrated that the ASF-shortened ribosomes have higher translocation activity than the WT ribosome. These observations indicate that the intrinsic translocation activity of ribosomes is greater than that usually observed in the WT ribosome and that ASF is a functional attenuator for translocation that serves to maintain the reading frame.

Ribosomes are universally conserved ribonucleoproteins that translate the genetic information contained in mRNAs into proteins. In bacteria, the large (50 S) and the small (30 S) subunits associate to form functional 70 S ribosomes. Both subunits are connected by 12 intersubunit bridges formed by RNA, protein-RNA, and protein-protein interactions (1, 2). After peptide bond formation, elongation factor G (EF-G) binds to the aminoacyl (A)-site of the ribosome and catalyzes the translation of the peptidyl-tRNA along the mRNA from the A-site to the peptidyl (P)-site. During this step, the head of the 30 S subunit undergoes a ratchet-like rotation relative to the 50 S subunit (3). Moreover, bridges B1a and B1b, which join the central protuberance (CP) of the 50 S to the head region of the 30 S subunit, undergo large conformational changes.

Helix 38 (H38, nucleotide positions (np) 827–942), which is located in domain II of 23 S rRNA, is a long helical structure that protrudes into the intersubunit space from the base of the CP in the 50 S subunit (Fig. 1). It participates, along with protein S13 in the 30 S head region, to form bridge B1a (1). H38 is widely conserved in eubacteria, archaea, and eukaryotes and is known as the “A-site finger (ASF)” (4) since it is located just above the A-site tRNA and interacts directly with the elbow region (D and T loops) of A-site tRNA through decoding (1, 5, 6). The 5.5-Å structure of the Thermus thermophilus ribosome (1) shows that the D and T loops of the A-site tRNA interact with the ASF at np 881–883 and np 898–899, respectively. Moreover, np 886–888 (Fig. 1A) at the tip of the ASF interact with amino acid residues 92–94 in S13 (1). However, in the 3.5-Å structure of the Escherichia coli ribosome (7), bridge B1a is not visible because of a disorder of the tip of the ASF maybe arising from the crystallization process. During the ratchet-like rotation that occurs upon EF-G binding (3), the ASF changes its binding partner to S19 instead of S13 (8). The computer simulation of this process (9) has suggested that this dramatic motion of the ASF is due to the kink-turn motif in its base region. Notably, the binding of the EF-Tu-GTP-aminoacyl-tRNA ternary complex does not affect the conformation of the ASF (10). It is known that the ASF also interacts with RF3 after the dissociation of RF1/2 (11).

Despite the many structural and biochemical analyses of the ASF and bridge B1a and their participation in the elongation cycle that have been described above, the functional role of the ASF remains unclear. To define the functional importance of the ASF, we constructed ribosome variants whose ASF is shortened. Biochemical characterization of these ASF-shortened ribosome provided clear evidence that indicates the ASF is...
**Truncation of Helix 38 in 23 S rRNA Elevates Translocation Activity**

involved in translocation. On the basis of these observations, we discuss the likely functional role the ASF plays in translocation.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Cultivation—**E. coli Δ7rrn strain TA542 (ΔrnrE ΔrnrB ΔrnrA ΔrnrH ΔrrnG::cat ΔrrnC::cat ΔrrnD::cat ΔrecA56/pTRNA66 pHKrrnC) (12) was kindly provided by Dr. Catharine L. Squires (Tufts University). The rescue plasmid pRB101 (13) was constructed by introducing the SacB gene and *rrnB* operon into pMW118 (Amp') (Nippon gene). The plasmid pRB102 (13) was constructed from pMW218 (Km') (Nippon gene) by inserting the *rrnB* operon only. The plasmid pHKrrnC in strain TA542 was replaced by pRB101 to generate strain NT101, which was used as the host cell to construct variant strains in this study. Cells were grown at 37 °C in 2× Luria-Bertani (LB) (2× LB, 2% tryptone, 1% yeast extract and 1% NaCl) medium; for solid medium, 1.5% agar was added. Antibiotics were added at the following concentrations when required: 40 μg/ml spectinomycin, 100 μg/ml ampicillin, and 50 μg/ml kanamycin. To replace the plasmid in NT101, 5% sucrose was added to the LB medium. NT102 is a series of *E. coli* strains in which pRB101 has been replaced by pRB102 or its derivatives. A precurture aliquot (A_mrn = −1.0 to −1.2) was inoculated into 1.5 ml of 2× LB medium. After being separated into five multiple aliquots, the growth rate of the NT102 variants was determined by measuring the A_mrn every 30 min by using a Spectramax 190 plate reader (Molecular Device, Inc.).

**Construction of Plasmids Generating 23 S rRNA with Shortened Helices 38, 34, or 68 and Variant Strains—**The template plasmid pRB102 was hypermethylated by M-AluI, M-HaeIII, and M-HapII as described (13) and subjected to QuikChange site-directed mutagenesis (Stratagene) according to the manufacturer’s instructions. For this, these following primer sets were used: H38d22 fwd: cactgttctcgggagaccttgaaacttggaatacgggag (forward) and gttgcatcttcggcgaactgtctgtactcctgattcccccgggag (reverse); H38d34, ctccggggtgagcacttgtctgtactcctgattcccccgggag (forward) and cattcttcggggtgagcacttgtctgtactcctgattcccccgggag (reverse); H34d, agttggaaggttgatctgctgggagacctgtctgtctgattcccccgggag (reverse); H34d, agttggaaggttgatctgctgggagacctgtctgtctgattcccccgggag (forward) and gttgcatcttcggcgaactgtctgtactcctgattcccccgggag (reverse); H63d, gtcgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgctgct
activity was measured by using o-nitrophenyl-β-galactopyranoside as a substrate according to the literature (21).

Translocation Assay—Toeprinting assays were used to determine the extent of translocation (22). Ribosomes (400 nM), tRNAfMet (800 nM), and ASL6Phe (6.0 μM) were prepared individually in buffer containing 80 mM potassium cacodylate (pH 7.2), 20 mM MgCl2, 150 mM NH4Cl, and 3 mM 2-mercaptoethanol. The ribosomes were activated and preprogrammed as described previously (23, 24). ASL6Phe was then added to the preprogrammed ribosome mix and A-site binding was allowed to occur for 30 min at 37 °C, followed by 20 min on ice. Sample mixtures were left at room temperature for 10 min. EF-G and GTP were prepared in binding buffer and added (final concentrations of 80 nM and 2 μM per reaction, respectively) to bring the final reaction volume to 25 μl. Sample mixtures were incubated with EF-G at room temperature. 2-μl aliquots were removed and placed on ice before the addition of EF-G for a background translocation control, and at each time point thereafter (30 s and 2, 5, 10, 30, and 60 min). Avian myeloblastosis virus-reverse transcriptase was added to each aliquot and primer extension was carried out at 37 °C for 10 min. Extension products corresponding to the pre- and post-translocation complexes were separated on a 10% denaturing polyacrylamide gel and visualized by autoradiography. A PhosphorImager (GE Healthcare) was used to quantify the intensity of the bands seen on the gel. Total counts within each lane were the sum of the counts for the pre- and post-bands. The fraction of translocated ribosomes is equal to the counts for the post-band divided by the total counts. All toeprint data shown are the result of at least three independent experiments.

RESULTS

Construction of Ribosome Variants with Shortened Bridge-forming Helices—To analyze the functional importance of the ASF in 23 S rRNA, we employed E. coli strain NT101, which lacks all chromosomal rRNA operons and is rescued by the plasmid pRB101, which harbors the rrnB and sacB genes (13, 14). Manipulation of the bridge-forming helices in 23 S rRNA was carried out on plasmid pRB102, which encodes rrnB and has the same replication origin as pRB101 but bears a different selection marker. We constructed ribosome variants with shortened bridge-forming helices, namely, H38/ASF, H34 and H68, which form the B1a, B4, and B7a bridges, respectively (Fig. 1A). The deletions were intended to disrupt each intersubunit bridge. Truncation of Helix 38 in 23 S rRNA Elevates Translocation Activity
by 47 bases (ΔA1845–1895, GGAA insertion). NT101 was transformed with each pRB102 variant and selected by kanamycin and sucrose. Each plasmid displaced the rescue plasmid pRB101 to generate cells showing sucrose resistance and ampicillin sensitivity. The resultant strains can thus survive with only a ribosome variant with a shortened rRNA helix. This indicates that the various deletions in the bridge-forming helices did not abolish minimal ribosomal function.

A Shortened ASF Does Not Affect Subunit Association—The growth rate of H38d22 and other strains was measured (Table 1). Judging from the doubling time, each deletion in the bridge-forming helix did affect the growth rate to some extent. As shown in Table 1, H38d22 showed only a small reduction in the growth rate (58.6 min versus 52 min for the WT strain), whereas the H68d variant showed a severe growth defect (108 min). The H38d34 and H34d variants showed intermediate reductions in the growth rate (67.1 and 61.7 min, respectively). Thus, the mutations in the intersubunit bridges decreased ribosome functionality, which indicates that these bridges do play some functional roles.

The efficacy of subunit association in each ribosome variant was measured by SDG centrifugation. The association ratio was determined by measuring the amounts of 70 S and free 50 S. The total 50 S levels were calculated by adding the height of the 50 S peak to the 50 S fraction in the 70 S peak. The association ratio was then obtained by dividing the 50 S fraction in 70 S by the total 50 S levels.

### Table 1

| Strains   | WT | H38d22 | H38d34 | H34d | H68d |
|-----------|----|--------|--------|------|------|
| Doubling time (min) | 52.1 ± 1.6 | 58.6 ± 3.5 | 67.1 ± 1.6 | 61.7 ± 0.84 | 108 ± 1.2 |
| Association ratio | 0.71 | 0.70 | 0.67 | 0.40 | 0.54 |

### Table 2

| Strains   | WT | EP | HF | H38d22 | H38d34 |
|-----------|----|----|----|--------|--------|
| Frameshift | 3.5 | 169 | 25 | 760 ± 25 | 827 ± 31 |
| Read-through | 3.9 | 872 | 3.3 | 39.3 | 68.5 ± 5.7 |

### Table 3

| Strains   | WT | EP | HF | H38d22 | H38d34 |
|-----------|----|----|----|--------|--------|
| GTPase activity | 3.7 | 117 | 3.3 | 39.3 | 68.5 ± 5.7 |

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**Truncation of Helix 38 in 23 S rRNA Elevates Translocation Activity**

**A Shortened Ribosomes Exhibit Enhanced Translation Activity at Limited EF-G Concentrations**—During EF-G-catalyzed translocation, bridge B1a becomes disrupted and the relative orientation of the ASF and S13 alters significantly (8). To further determine the function of the ASF in translation, we examined the translational activity of the ASF-shortened ribosome H38d22 in an *in vitro* translation reaction with varying concentration of EF-G (0–125 nM). The initial velocity of polyuridine (poly(U))-dependent polyphenylalanine (poly(Phe)) synthesis of each ribosome variant is plotted in Fig. 2A. H38d22 showed higher poly(U)-poly(Phe) synthesis than the WT but only at limited EF-G concentration. In contrast, both the H34d and H68d variants showed lower activity than the WT throughout the full range of EF-G concentration. At higher EF-G concentration (200–600 nM), H38d22 and WT showed equivalent translational activity (data not shown, see Fig. 2B). Moreover, with regard to poly(U)-poly(Phe) synthesis in the presence of sufficient levels of EF-G (600 nM) and varying concentrations of EF-Tu (Fig. 2B), H38d22 showed similar translational activity to the WT. We also found the H38d34 variant had higher translational activity than the WT (Fig. 2C), although to a lesser degree than H38d22. Thus, ASF truncation enhances translation activity at limited EF-G concentration.

The GTPase activity of EF-G is known to be highly stimulated by vacant ribosomes (27, 28). We thus next examined the ability of each ribosome variant to stimulate EF-G-related GTPase activity. As shown in Fig. 2D, the initial GTPase activity was elevated more efficiently by H38d22. In contrast, the H34d and H68d variants showed a comparable GTPase activity with WT ribosome.

**ASF Truncation Stimulates Translocation**—Since the higher translational activity of the ASF-shortened ribosome was
observed only at limited EF-G concentrations, we next assessed the single round translocation of the ASF-shortened ribosomes compared with the WT ribosome. Prior to performing the translocation assay, we assessed the tRNA binding and peptidyl transfer activity of H38d22. Non-enzymatic binding of phenylalanyl (AcPhe)-tRNA\textsuperscript{Phe} to the P-site (Fig. 3B). Then, puromycin was added to estimate peptidyl transfer reaction of H38d22. About 20% of AcPhe was released from the ribosome as AcPhe-puromycin in this reaction. By counting AcPhe-puromycin released from the ribosomes, no significant difference in the reactivity of puromycin was observed (Fig. 3C). These results revealed that H38d22 has similar activities for tRNA binding and peptidyl transfer reaction to the wild type ribosome.

We next measured the translocation activity of the ASF-shortened ribosome by performing a toeprinting assay. In this assay, the P-site is prefilled with deacyl-tRNA\textsubscript{fMet} and the A-site is occupied by the anti-codon stem loop analog, ASL6\textsubscript{Phe}. Preprogrammed ribosomes were incubated with EF-G to induce translocation or without EF-G as a control. The pre- and post-translocation mixes were subjected to primer extension analysis to measure the distance between the ribosome and the primer, which hybridizes to the downstream point of the mRNA. As shown in Fig. 4, both H38d22 and H38d34 showed faster translocation than the WT ribosome in the presence of both low (Fig. 4A) and high (Fig. 4B) concentrations of EF-G. While the initial velocity of translocation for both ASF-shortened and WT ribosomes was accelerated by high concentrations of EF-G (Fig. 4B), the enhanced activity of the ASF-shortened ribosomes was maintained. These results suggest that ASF truncation enhances translocation at each step. Taken together with the poly(U)-poly(Phe) synthesis results (Fig. 2A), it appears that H38/ASF in the WT ribosome acts as a negative regulator of EF-G turnover during translocation.

**DISCUSSION**

H38/ASF in 23 S rRNA is a component of the intersubunit bridge B1a. In this study, we analyzed the role H38/ASF plays in translation by constructing *E. coli* strains that bear the ASF-shortened ribosomes. We found these strains were all healthy and had a normal growth rate. They did show moderate frameshift activity, but the ASF truncation did not affect subunit association. This was unlike the other ribosomes with shortened H34 or H68 bridge-forming helices, which showed reduced association. These observations suggest that the ASF and bridge B1a are unlikely to play an important role in subunit association, rather, they probably have another function in translation.

![Figure 2. In vitro translation and EF-G-related GTPase stimulatory activity of ribosome variants with shortened bridge-forming helices. A–C, initial velocity of poly(U)-poly(Phe) synthesis. In A, the EF-G concentrations were titrated and EF-Tu was present at 600 nM. In B, the EF-Tu concentrations were titrated and EF-G was present at 600 nM. In C, the productivity of poly(U)-poly(Phe) synthesis of ASF-shortened ribosomes performed with 10 nM EF-G for 10 min was determined. D, EF-G-related GTPase activation stimulated by ribosome variants with shortened bridge-forming helices. The P\textsubscript{i} released from [γ\textsuperscript{32}P]GTP was resolved by TLC and quantified by a fluor imager (Fujifilm BAS5000). The WT and H34d, H68d, and H38d22 variants are represented by black squares, white squares, black circles, and white circles, as in A and B.](image1)

![Figure 3. A- and P-site tRNA-binding and reactivity to puromycin by ASF-shortened ribosomes. Shown are the A-site tRNA-binding (A), P-site tRNA binding (B), and peptidyl transfer (C) activities of WT and H38d22 ribosomes. The error bars indicate S.D. values.](image2)

![Figure 4. Translocation activity of H38d22 and H38d34 ribosomes. A–C, translocation activity of H38d22 and H38d34 ribosomes compared with WT ribosomes. The ribbon gel shows the mobility of the ribosomes on a native gel. The ribosomes were incubated with EF-G at 100 nM and EF-Tu at 600 nM. The distance between the ribosome and the primer, which hybridizes to the downstream point of the mRNA, was measured by primer extension analysis.](image3)

Truncation of Helix 38 in 23 S rRNA Elevates Translocation Activity
at limited concentration, although both translate equally well when more EF-G (200–600 nM) is present. This implies that the ASF-shortened ribosome interacts better with EF-G during the elongation cycle or turns it over more quickly than the WT ribosome. Supporting this is that H38d22 stimulated EF-G-related GTPase activity significantly better than WT ribosome, although its molecular event is different from the case in actual translocation. Single round translocation followed by toeprinting analysis then clearly demonstrated that the ASF-shortened ribosomes show greater translocation activity than WT ribosome even when high concentrations of EF-G (2 μM) were present.

Notably, at this EF-G concentration, ASF-shortened and WT ribosomes did not differ in an in vitro translation activity (data not shown). These observations together suggest that the more efficient translation of the ASF-shortened ribosomes at limited EF-G concentration is due to a more rapid turnover of EF-G. As EF-G and the ASF have not been observed to interact directly upon EF-G binding (3, 8), a plausible interpretation of our data is that truncation of ASF results in a lowering of the energy required to convert a ribosome from a pre-translocation to a post-translocation state. Furthermore, we found that although the ASF interacts with the elbow region of A-site tRNA (1), ASF-truncation did not affect A-site tRNA binding. In addition, H38d22 showed normal P-site tRNA binding and peptidyl transfer activity. These observations together thus suggest that H38/ASF is mainly involved in EF-G-catalyzed translocation. However, since the ASF-shortened ribosomes showed +1 frameshift activity, it is possible that the truncation of the ASF may also affect tRNA positioning during translocation on the ribosome. Nevertheless, the observations in this study provide evidence that the intrinsic potential of translocation is actually greater than is observed in the WT ribosome, which indicates the presence of a negative regulatory mechanism. Thus, H38/ASF appears to be a functional region that acts as a negative regulator of translocation.

Dontsova and colleagues (25) have recently also characterized the effect of truncating the ASF (Δ872–905). They observed that their ASF truncation, which is more extensive than ours (34 bases versus 22 bases in our study), led to growth defects and less efficient subunit association. In addition, their ASF-shortened ribosome showed no difference in EF-G-related GTPase stimulation and translocation activity. The discrepancy between these results and ours may be due to several reasons. First, our genetic system is different from theirs. They used an E. coli strain lacking all chromosomal rrn operons but bearing a multicopy plasmid (ColE1 origin) containing the rrnB operon under the control of P_l promoter. We used a strain (TA542 derivative) that also lacked all chromosomal rrn operons but that was rescued by a low copy number plasmid (pSC101 origin) containing the rrnB operon under the control of P1P2 promoter. Given that E. coli has seven rrn operons in its genome, the complementation of the rrn operon with its original promoter by using a plasmid with a pSC101 origin may result in wild type copy numbers of the operon in the cell. Supporting this is that the doubling time of our strain (about 50 min) is somewhat shorter than that of theirs (87 min). Second, as mentioned above, the ASF of Dontsova and colleagues (25) had a longer truncation. When we constructed ribosomes bearing this longer truncation ourselves (H38d34), we found that our original mutant H38d22 had higher translocation activity while H38d34 ribosomes induced a lower growth rate (67.1 min) and a less efficient poly(U)-poly(Phe) activity at a limited EF-G concentration (Fig. 2C). However, H38d34, like H38d22, also showed efficient single round translocation (Fig. 4). Thus, despite the variations in phenotype induced by the truncation
of the ASF by 34 as opposed to 22 bases, our observations indicate that ASF-truncation in general enhances translocation.

It has been observed that when ribosomes are treated with the thiol-specific reagent pCMB (p-chloromercuribenzoate), spontaneous translocation occurs (29, 30), which suggests that the ribosome itself has all the equipment needed for translocation. The ribosomal protein S12 in 30 S was proposed to be the target of pCMB (31, 32), and indeed, Green and colleagues (33) showed that ribosomes depleted of S12 and/or S13 exhibit spontaneous translocation. Since S13 interacts with H38/ASF to form the B1a bridge, and since S13 depletion also disrupts bridge B1a, the molecular mechanism that allows the ASF-shortened ribosome to undergo faster translocation may be related somehow to the mechanism involved in spontaneous translocation. However, since the ASF and S13 both have other ribosomal functions, this relationship cannot be easily elucidated.

In eukaryotes, H38 is longer and forks into two stems, while the forked stem-loop is located in the back (solvent side) of the CP, the major stem-loop of H38 interacts with the A-site and forms bridge B1a, similar to what is found in eubacteria (34). Thus, bridge B1a and the ASF are broadly conserved in eu- bacteria, archaea and eukaryotes. In animal mitochondrial ribosomes, on the other hand, H38 is very short and cryo electron microscopy of the bovine mitochondrial ribosome has failed to detect bridge B1a (35). Instead, the mitochondrial ribosome has another structural protrusion named the P-site finger (PSF) near the P-site region. Since ratchet-like rotation upon EF-G binding is not observed in the bovine mitochondrial ribosome (Agrawal, personal communication), translocation in mammalian mitochondrial ribosomes may involve distinct molecular mechanisms.

The swiveling of the head region of 30 S that was observed in the two crystal structures of E. coli ribosomes (7), coupled with the ratchet-like rotation that was observed to occur upon EF-G binding in cryo-EM structures (3), has revealed a part of molecular mechanisms of translocation. Analysis of the two structures that comprise the pre- and post-translocation state shows that this structural change is associated with the event that the ASF alters its interacting partner from S13 to S19. Our biochemical observations described here suggest that ASF-truncation seems to cause the looseness of this partner switch as well as EF-G positioning, thereby accelerating translocation. Consequently, we propose that the ASF acts as a functional attenuator that regulates translocation for the purpose of maintaining translational fidelity.

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