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# Traveling Time of a Translating Ribosome along Messenger RNA Monitored Directly on a Quartz Crystal Microbalance

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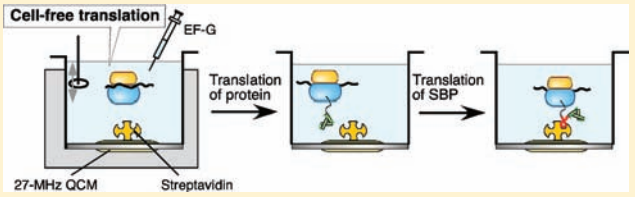
Supporting Information

**ABSTRACT:** During translation, the biosynthesis of polypeptides is dynamically regulated. The translation rate along messenger RNA (mRNA), which is dependent on the codon, structure, and sequence, is not always constant. However, methods for measuring the duration required for polypeptide elongation on an mRNA of interest have not been developed. In this work, we used a quartz crystal microbalance (QCM) technique to monitor mRNA translation in an *Escherichia coli* cell-free translation system in real time. This method permitted us to evaluate the translation of proteins of interest fused upstream of a streptavidin-binding peptide (SBP) fusion protein. The translation of mRNA encoding the SBP fusion protein alone was observed as a mass increase on a streptavidin-modified QCM plate. Addition of the protein of interest resulted in a delay in the mass change corresponding to the traveling time of the ribosome along the coding region of the protein of interest. With this technique, the lengths of coding sequences, codon usages, influences of unique sequences, and various protein-coding sequences were evaluated. The results showed that the traveling time of the translating ribosome depends on the length of the coding region translated but is also affected by the sequence itself. Differences in the time lags for various proteins imply that mRNA coding sequences may regulate gene expression.




## INTRODUCTION

The levels of gene expression can be regulated not only during transcription by RNA polymerase but also during translation by ribosomes. The translation process is divided into three steps: translation initiation, elongation, and termination.[1−3] The elongation step is the most important in that the ribosome scans, decodes the messenger RNA (mRNA) coding region, and translates it to a polypeptide. This step is performed with the help of translation elongation factors (EFs). In bacteria, EF-Tu and EF-G mainly participate in the reaction. EF-Tu forms ternary complexes with aminoacyl transfer RNA (tRNA) and GTP to deliver aminoacyl-tRNA into the A site of the translating ribosome. The peptidyl transferase reaction occurs after the correct amioacyl-tRNA is delivered into the A site. EF-G, which is a G-protein, binds to the A site after peptide formation and catalyzes translocation to induce movement of the ribosome to the next codon. During the elongation process, however, the rates at which individual codons are translated are quite different.[4] First, when the ribosome translates a rare codon, the translation rate decreases, because the amount of the corresponding tRNA is also small in the host cell. It was recently reported that silent polymorphisms cause mutations that generate major codons from rare ones, which in turn affect the folding of the protein.[5−7] Second, some specific nascent polypeptides such as SecM and TnaC interact with the exit tunnel of the ribosome to stall the translating ribosome on the mRNA.[8−10] The secondary structure of mRNA influences translation elongation to cause an abnormal translation stall.[11] To elucidate the mechanism of cotranslational events, it is important to monitor the translation process. Some methodologies for monitoring translation have been reported. For example, reporter gene expression systems such as the luciferase gene have often been used to analyze the processivity of ribosomes along 5′-untranslated regions (5′-UTRs) and open reading frames.[12,13] Fluorescent proteins have also been utilized to measure the single-molecule translation of green fluorescent protein by total internal reflection fluorescence microscopy.[14] In these instances, however, monitoring requires the use of a specific reporter gene; also, enzyme activities are not always correlated with the amount of protein translated. Although an optical tweezers technique has been utilized to monitor single-molecule translation along a structured mRNA, only artificial mRNAs are analyzable.[15] Therefore, it has been difficult to evaluate the translation elongation rate along mRNAs of interest.

We recently developed a novel technique for measuring the translation of a streptavidin-binding peptide (SBP)−protein D−SecM fusion protein.[16] The technique uses a streptavidin-modified 27 MHz quartz crystal microbalance (QCM), which

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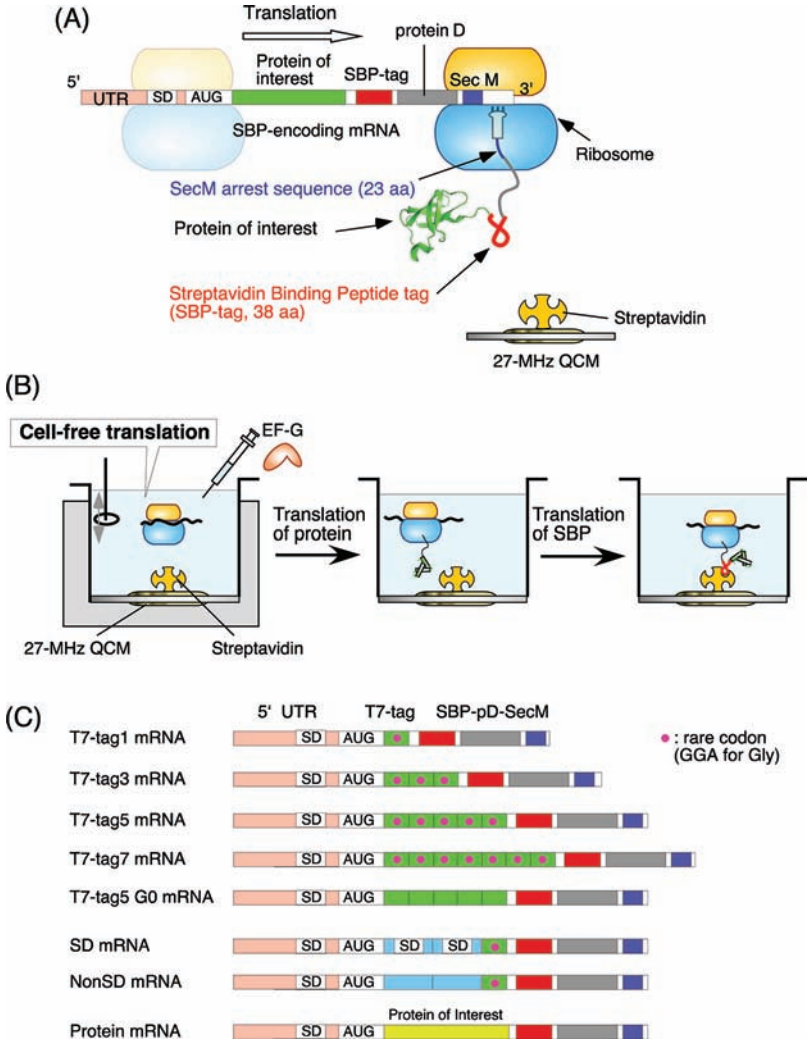


**Figure 1.** (A) Schematic illustration of the *E. coli* 70S ribosome–nascent chain complex (RNC) translating the protein of interest followed by the SBP tag (AUG, start codon; SD, Shine–Dalgarno sequence). (B) Trapping of the SBP tag of the RNC on a streptavidin-modified 27 MHz quartz crystal microbalance (QCM) triggered by the addition of EF-G. (C) Structures of the mRNAs used in this work.

enables us to evaluate a cell-free, single-turnover translation reaction with no labeling (Figure 1B).[16−18] A QCM is a very useful tool for analyzing enzymatic reactions in solution[19,20] because it can detect the change of a universal unit of mass on its surface at the nanogram level. Thus, we can measure translation because the translation reaction always coincides with a mass increase due to the generation of a nascent polypeptide chain. Here, in order to evaluate translation, the sequence of a gene of interest was incorporated upstream of the coding sequence of the SBP–SecM fusion protein (see Figure 1). As a result, the *Escherichia coli* 70S ribosome–nascent chain complex (RNC) could form. The time taken to generate the SBP-displayed ribosome is longer for a ribosome translating a protein of interest fused to SBP than for a ribosome translating SBP alone. Therefore, we can estimate the traveling time (the rate of polypeptide elongation) of a ribosome translating a protein of interest. This established method allowed us to assess the effects of length and codon usage on the rate of translation by measuring changes in the time course of mass increases for model mRNAs. In addition, the translation of various proteins of interest revealed that the time required for a ribosome to travel through the mRNA does not always depend on mRNA length but rather can depend on other factors such as codon usage or specific sequences that interact with the ribosome during translation.

## ■ EXPERIMENTAL SECTION

**Materials.** 1-Ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC) was purchased from Dojindo Laboratories (Kumamoto, Japan), and *N*-hydroxysuccinimide (NHS) and streptavidin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Total tRNA from *E. coli* MRE600 strain was purchased from Roche (Tokyo, Japan). The oligonucleotides were purchased from Operon (Tokyo, Japan). All other materials were purchased from Nacalai Tesque (Kyoto, Japan) and used without further purification.

**Preparation of mRNAs.** For cloning each complementary DNA (cDNA), we used a vector constructed previously[16,21] that encodes an SBP–protein D–SecM fusion polypeptide under the T7 promoter. For the construction of cDNAs with model sequences to observe the translation reaction, synthetic oligonucleotides (upper chain, 5′-TATGGCTAGCATGACTGGTGGACAGCAAATGGGTTC; lower chain, 5′-TAGAACCCATTTGCTGTCCACCAGTCATGCTAGC-CA) encoding the 11 amino acid (aa) T7 tag (MASMTGGQQMG) were annealed, phosphorylated, and inserted into the NdeI site positioned at the start codon of SBP on the vector to construct the T7-tag1 gene (encoding T7-tag–SBP–ProteinD–SecM). For construction of the vectors encoding $(T7\ tag)_n$ ($n$ = 3, 5, 7), we digested the generated vector with NdeI again and inserted a T7-tag-encoding


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cassette. Each cDNA of Shine–Dalgarno (SD) mRNA (ATGGCTAGCATGACTCTCACGCTCACAAGGAGGGCTAGCGCAACTGGTGGACAGCAAGCTGGTTCT) or non-SD mRNA (ATGGCTAGCATGACTCTCACGCTCACACGCCGCGCTAGCGCAACTGGTGGACAGCAAGCTGGTTCT) was made from synthetic oligonucleotides, and two copies were ligated in front of SBP as described above. To construct cDNAs encoding native protein sequences, the genes for *CspA*, *IF1*, *NirD*, *HyaE*, and *MutH* were amplified by direct polymerase chain reaction (PCR) from a colony of *E. coli* JM109 and inserted within the NdeI site as above. *DHFR* was amplified from the plasmid for the expression of DHFR.[22] *CAT* was amplified from the pACYC184 plasmid. The template DNAs for in vitro transcription were amplified by PCR as previously described,[16] after which mRNA was transcribed using the CUGA7 in vitro transcription kit (Nippon Gene) and purified with MicroSpin G-25 columns (GE Healthcare).

**Preparation of tRNAs.** *E. coli* Rosetta(DE3) strain (Novagen) was cultivated on a Luria–Bertani (LB) broth agar plate containing 30 μg/mL chloramphenicol (Cm) at 37 °C overnight. An isolated colony was picked and cultured in 6 mL of LB broth with Cm at 37 °C overnight. The culture was transferred into 2 L of 2× YT medium with Cm at 37 °C for 20 h. The cultures were harvested by centrifugation and resuspended in 20 mL of 1 mM Tris-HCl (pH 7.2) containing 10 mM $MgCl_2$. Phenol (20 mL) saturated with Milli-Q water was added, and the tube was vigorously shaken. The mixture was centrifuged at 4 °C for 30 min at 10000*g*. The supernatant of the aqueous phase was collected and combined with 1/10 vol of 5 M NaCl and 2 vol of ethanol. The mixture was centrifuged at 4 °C for 30 min at 10000*g*. The precipitate was resuspended in 10 mL of 1 M NaCl and spun at 4 °C for 30 min at 10000*g*. The supernatant was collected and precipitated with ethanol. The pellet was dissolved in 4 mL of 1.8 M Tris-HCl (pH 8.0) for 90 min at 37 °C. After that, 0.4 mL of 5 M NaCl and 8.8 mL of ethanol were added, and the solution was centrifuged. The pellet was rinsed once with 70% ethanol, dried, and resuspended in 15 mL of buffer A [20 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, 0.1 mM EDTA, 0.2 M NaCl]. The extract of tRNA was loaded onto the Hitrap Q HP column (GE Healthcare). Thereafter, the tRNA fraction was recovered from the fraction eluted with a NaCl gradient. The pooled mixture was precipitated with ethanol and dissolved in 2 mL of Milli-Q water.

The genes encoding $tRNA^{Gly}$ (*glyT*) and $tRNA^{Arg}$ (*argW*) were cloned from the *E. coli* XL-1 Blue strain between the *Eco*RI and *Pst*I sites in the pBStRNALfMet vector.[23] *E. coli* XL1-Blue strain was transformed with the constructed vectors. Fresh transformants were cultivated, and each overexpressed tRNA was purified as described above for Rosetta tRNA.

**Translation in a QCM cell.** All of the translation factors for the cell-free mixture (PURESYSTEM) were prepared and purified as described previously.[16−18] The procedures for setting up the QCM system and calibration of the 27 MHz QCM in buffer solutions were basically the same as those reported previously.[16] The translation reaction mixture was prepared as shown in Table S1 in the Supporting Information. Prior to measurements, 50 μL of the enzyme mixture, which contained the 70S ribosomes, initiation factors (IFs), EFs, aminoacyl-tRNA synthetases, and methionyl tRNA formyl transferase (MTF), was incubated at 37 °C for 5 min as a preincubation mixture. The enzyme mixture was then added to 450 μL of the reaction mixture, which contained all of the enzymes except the ribosomes, together with the substrates and chemicals, in the QCM cell (volume, 500 μL) in the QCM apparatus at 25 °C. After the frequency became constant (within 2 Hz/s), the solution of EF-G was injected to start translation 20 min after the mRNA was injected. The reaction mixture was stirred vigorously to avoid any anomalies due to lack of mixing, and the decreases in frequency (increases in mass) were followed over time. The traveling time of ribosomes along mRNA was calculated from the time lag to a decrease in frequency (increase in mass) on the streptavidin-modified QCM.[16] The translation rate was calculated by subtracting the time lag for SBP–protein D–SecM (7.9 ± 0.23 min with MRE600 tRNA or 6.1 ± 0.12 min with Rosetta tRNA) from the time lag obtained for the protein of interest and dividing the length of the protein (number of amino acids) by the difference calculated. The $\Delta G$ values for mRNAs were obtained from the web server of MFOLD.[24]

## RESULTS AND DISCUSSION

**Analysis of Traveling Times of Ribosomes.** First, the translation of the T7 tag (from T7 phage gene 10) was measured as a model experiment. The T7 tag, which encodes the 11 aa sequence MASMTGGQQMG, has been broadly used as a tag for immunoassays and affinity purification when conjugated to a protein at the N- or C-terminal or placed internally. It did not show any nonspecific effect on translation in the QCM system.[16] Here we constructed a series of mRNAs containing a tandem repeat of one, three, five, or seven T7 tags (denoted as T7-tag*n*, $n$ = 1, 3, 5, 7, respectively) as the protein of interest upstream of the SBP (Figure 1C). The QCM instrument used in this work, calibrations of the QCM, preparation of mRNA, and QCM experimental procedures were the same as described previously except for a slight modification[16] (see Table S1 in the Supporting Information). To estimate the exact time of polypeptide elongation, we had to exclude the time required for translation initiation because the process of translation initiation is complicated and formation of the initiation complex is time-consuming.[25] Thus, we prepared a cell-free mixture without EF-G. In this system, ribosomes in the QCM cell were not able to proceed with peptide elongation but could form an initiation complex, which excluded the time for translation initiation from subsequent measurements. After this, EF-G was added to restart the translation (Figure 1B).

Figure 2 shows typical changes in frequency as a function of time after the injection of EF-G, illustrating how frequency decreases depend on the length of the tandem repeat of T7 tags upstream of the SBP tag in the mRNA. Frequency decreases reflect mass increases on the QCM that result from binding of the SBP tag to the streptavidin-modified QCM after translation through different numbers of T7 tags. When T7-tag1 mRNA was employed, the frequency decreased (the mass increased) gradually after a time lag of 8.5 min and finally reached a constant value of $\Delta F = -1500$ Hz ($\Delta m = 280$ ng cm$^{-2}$; curve a). The time lag after injection of EF-G to trigger translation reflects the duration of translation from the tandem repeats of the T7 tag through the SBP tag. Since the length of the ribosome exit tunnel is ∼90 Å,[26,27] the time lag in fact indicates the time at which the first internal SBP tag emerges from the tunnel after translation of at least 30 aa downstream of the SBP-coding region. As the number of incorporated T7 tags increased, the time lags clearly became longer, indicating that ribosomes took more time to travel along the tandem repeat of T7 tags. The time lags obtained were plotted against the lengths of the T7-tag-repeat coding regions, as shown in Figure 3. The results are summarized in Table 1.

There was a good linear relation between the time lag and the length of the coding region in the presence of tRNAs from the MRE600 strain (line a in Figure 3). Thus, the ribosome translated evenly along the tandem repeat of T7 tags. The traveling rate was calculated from the slope of line (a) to be 17 aa min$^{-1}$. This rate is relatively low compared with the rate of elongation in an *E. coli* cell (60–600 aa min$^{-1}$), mainly because of the relatively low temperature of 25 °C and the properties of the reconstituted cell-free mix used here.[28] The intercept of the regression line indicates that the traveling time of the ribosome for translation of the SBP–protein D fusion protein was 7.9 ± 0.23 min. Therefore, the exact traveling time along the T7 tags


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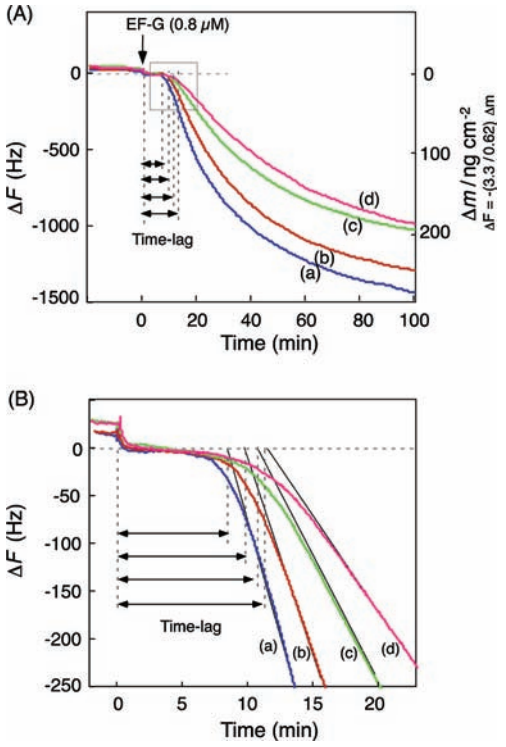


**Figure 2.** (A) Typical frequency decreases $\Delta F$ (corresponding to mass increases $\Delta m$) of the streptavidin-modified 27 MHz QCM cell in response to the addition of EF-G into the *E. coli* cell-free translation mixture. The coding regions of mRNAs added in advance contained a tandem repeat of one, three, five, or seven T7 tags as the protein of interest upstream of the SBP tag, as shown in Figure 1C. These employed mRNAs were (a) T7-tag1, (b) T7-tag3, (c) T7-tag5, and (d) T7-tag7 mRNA, respectively. (B) Enlargement of the initial slopes in Figure 2A. The assays were carried out in a cell-free mixture with tRNAs from the MRE600 strain in 50 mM HEPES-KOH (pH 7.6) containing 100 mM potassium glutamate, 6 mM $Mg(OAc)_2$, 2 mM spermidine, and 1 mM dithiothreitol (DTT) at 25 °C.

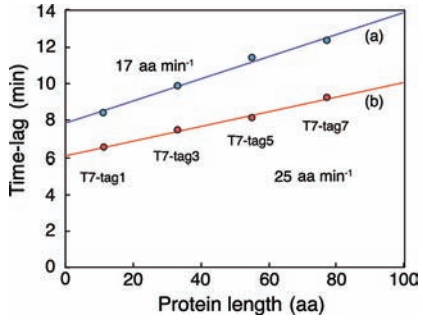


**Figure 3.** Relationship between the time lags obtained from QCM experiments and the length (in aa) of the tandem repeats of the T7 tag in T7-tag*n* mRNA ($n$ = 1, 3, 5, 7) in the presence of tRNAs from the (a) MRE600 and (b) Rosetta strains. The rates of translation determined from the slopes were 17 aa $min^{-1}$ with MRE600 tRNA and 25 aa $min^{-1}$ with Rosetta tRNA.

could be estimated by subtracting 7.9 ± 0.23 min from each value obtained.

**Effect of Rare Codons on the Translation of T7 Tags.** Cells contain low-usage codons called rare codons. When a translating ribosome encounters a rare codon in the mRNA,[29] the rate of translation slows because the concentration of the corresponding tRNA is notably low. The native T7 tag

**Table 1. Time Lags for Translation on mRNAs Encoding Proteins of Different Lengths and Varieties**[a]

| mRNA | $L$ (aa)[b] | added tRNA | time lag (min) MRE600 tRNAs | time lag (min) Rosetta tRNAs |
|---|---|---|---|---|
| T7-tag1 | 11 | | 8.5 ± 0.15 | 6.6 ± 0.29 |
| T7-tag3 | 33 | | 9.9 ± 0.25 | 7.5 ± 0.49 |
| T7-tag5 | 55 | | 11.5 ± 0.17 | 8.2 ± 0.20 |
| T7-tag7 | 77 | | 12.4 ± 0.18 | 9.3 ± 0.87 |
| T7-tag5 | 55 | $tRNA^{Gly}$ | 8.5 ± 0.55 | |
| SD mRNA | 55 | | 14.8 ± 0.49 | |
| SD mRNA | 55 | $tRNA^{Arg}$ | 13.1 ± 0.01 | |
| non-SD mRNA | 55 | | 9.5 ± 0.20 | |
| CspA | 66 | | 11.4 ± 0.2 | 8.13 ± 0.35 |
| IF1 | 72 | | 14.6 ± 1.5 | 13.4 ± 0.65 |
| NirD | 114 | | 19.0 ± 0.41 | 13.4 ± 0.53 |
| HyaE | 134 | | 17.5 ± 2.22 | 14.2 ± 0.48 |
| DHFR | 157 | | 15.7 ± 0.35 | 10.0 ± 0.44 |
| CAT | 219 | | 20.7 ± 1.0 | 11.7 ± 1.5 |
| MutH | 234 | | 33.1 ± 1.6 | 23.2 ± 0.95 |

[a]All of the experiments were carried out in the cell-free mixture with 50 mM HEPES-KOH (pH 7.6) containing 100 mM potassium glutamate, 6 mM $Mg(OAc)_2$, 2 mM spermidine, and 1 mM DTT at 25 °C. [b]Length of the protein of interest.

sequence contains one rare codon, GGA, encoding Gly in the *E. coli* K12 strain. To investigate the effect of the rare codon on translation, total tRNA extracted from the Rosetta(DE3) *E. coli* strain was employed. This strain can express additional amounts of tRNAs corresponding to rare codons such as Gly, Arg, Pro, and Ile in *E. coli* K12.[30] We extracted total tRNA from the Rosetta strain to use in place of total tRNA from MRE600. As expected, the time lag for T7 tag production was smaller with Rosetta tRNA (Figure 4B) than with tRNA from MRE600 (Figure 4A). As shown by line (b) in Figure 3, the relationship between the time lag and the length of the coding sequence was also linear in the presence of Rosetta tRNAs, with a slope corresponding to a traveling rate of 25 aa $min^{-1}$, which is higher than the rate of 17 aa $min^{-1}$ in the presence of MRE600 tRNAs. The difference in the slopes of the two regression lines indicates the effect of the Gly rare codons within the T7 tags, and the difference in the intercepts indicates that there were other rare codons such as AGA within the SBP−protein D sequence. In the translation of T7-tag5 mRNA (which has five Gly rare codons) with MRE600 tRNAs, the time lag decreased from 11.5 ± 0.17 to 8.5 ± 0.55 min upon addition of $tRNA^{Gly}$. The latter time lag is comparable to that in the presence of Rosetta tRNAs (8.2 ± 0.20 min; Table 1). Thus, the lower translation rate of T7-tag5 mRNA due to the presence of five rare codons could be increased by the addition of Rosetta tRNAs or $tRNA^{Gly}$.

**Effect of AGG Codon Independent of Codon Usage.** We next analyzed mRNA containing a Shine−Dalgarno (SD)-like sequence (5′-AGGAGG) within the coding region. The SD sequence is usually located in the 5′-UTR region immediately upstream of a start codon (AUG) as a consensus sequence for prokaryotic translation initiation (Figure 1A).[31] Ribosomes recognize the sequence through simple hybridization of a complementary sequence, called the anti-SD sequence, in the 3′-terminus of the 16S ribosomal RNA (rRNA) on the 30S subunit. It has been suggested that the SD/anti-SD duplex formed in translation initiation would not dissociate after


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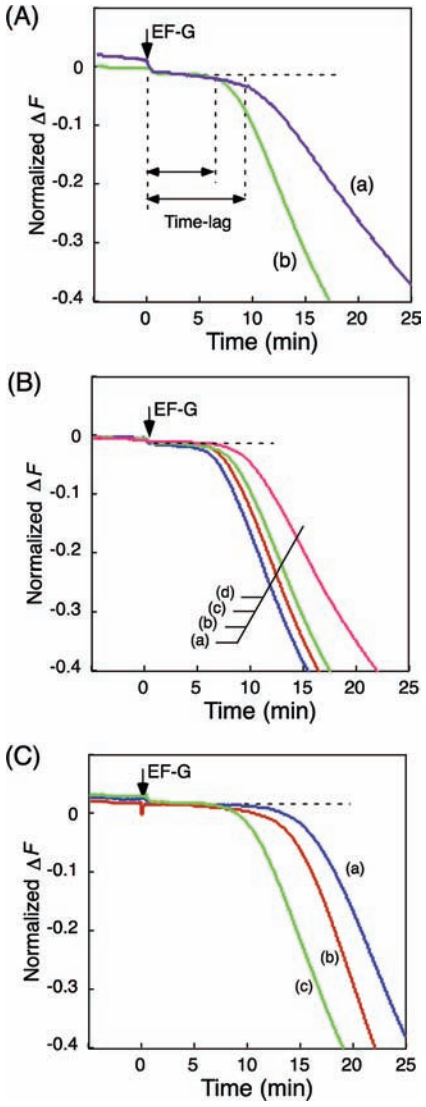


**Figure 4.** (A) Typical frequency decreases $\Delta F$ in response to the addition of EF-G to the streptavidin-modified 27 MHz QCM cell filled with an *E. coli* cell-free translation mixture containing T7-tag5 mRNA in the presence of tRNAs from the (a) Rosetta and (b) MRE600 strains. (B) Initial slopes of the frequency changes that occurred during translation with (a) T7-tag1, (b) T7-tag3, (c) T7-tag5, and (d) T7-tag7 mRNA in the presence of Rosetta tRNAs. (C) Initial slopes of the frequency changes that occurred during translation with (a) SD mRNA, (b) SD mRNA in the presence of $tRNA^{Arg}$, and (c) non-SD mRNA.

several rounds of decoding in order to control the stability of P-site tRNA for ribosome recycling.[32] The SD-like sequence within the coding sequence seems to be able to form a duplex with the anti-SD sequence of the ribosomal 30S subunit during translation to control translation frame shifting.[33,34] Here, to study the effect of an internal SD sequence on translating ribosomes, we designed mRNAs to encode a protein of the same length as T7-tag5 (55 aa). The mRNA had two repeats of 66 nucleotides (nt), each containing an SD sequence, fused in-frame with a single T7 tag (Figure 1C). The initiation of translation could not start near the internal SD sequence because the nearest AUG codon was 36 nt away, far enough from the internal SD sequence to prevent unintended formation of initiation complexes.[35] As shown by curve (a) in Figure 4C, SD mRNA showed relatively slow translation compared with non-SD mRNA lacking SD sequences. The time lag obtained from SD mRNA was 14.8 ± 0.49 min, which is longer than the time lag of 9.5 ± 0.20 min obtained from non-SD mRNA (Table 1). This is due to the strong interaction between the internal SD sequence and the anti-SD sequence of the ribosome.

The in-frame codon AGG within the SD sequence (AGGAGG) is also a rare codon, corresponding to Arg. Since there were no other AGG codons within the SBP−protein D−SecM sequence, $tRNA^{Arg}$ was added to investigate the effect of rare codons, as above. Although the time lag of 13.1 ± 0.01 min in the presence of $tRNA^{Arg}$ was 1.7 min smaller than the time lag without $tRNA^{Arg}$, it was still larger than the time lag of T7-tag5 mRNA in the presence of $tRNA^{Gly}$ (8.5 ± 0.55 min) because of the interaction with the anti-SD sequence. On the other hand, non-SD mRNA, which encoded ArgArg with the major codons CGCCGC instead of the rare codons AGGAGG, showed a much smaller time lag of 9.5 ± 0.20 min (Table 1). It is possible that the time lag of the SD mRNA was due to the effect of rare codons and the effect of the internal SD sequence, which slowed the processivity of the ribosome by interacting with the anti-SD sequence on the end of the 16S rRNA during translation. We evaluated the contribution of the codon usage and other sequence-specific factors to the traveling time of ribosome. The former was calculated from the difference in the time lag upon the addition of $tRNA^{Arg}$, and the latter was estimated from the difference between the time lag of SD mRNA in the presence of $tRNA^{Arg}$ and the time lag of non-SD mRNA. Interestingly, the effect of the internal SD sequence on the processivity of the ribosomes was stronger than the effect of the AGG rare codon at the concentrations of total tRNA used in this work.

**Translation of Sequences Encoding Native Proteins.** One of the greatest advantages of this technique is the ability to evaluate translation without using a reporter gene. Thus, we can investigate the traveling time of ribosomes along the coding regions of various proteins. As discussed above, the rate of translation along mRNA is regulated by the tRNA concentrations, the mRNA sequence, and the mRNA structure. Although the reason for variable rates of translation is still unclear, it is possible that sequence-specific information affecting the processivity of the ribosome "encodes" a way to regulate gene expression. Here we tested the efficiency of translation along the coding sequences of various proteins. We chose several genes from *E. coli* (*CspA*, *IF1*, *NirD*, *HyaE*, *DHFR*, *CAT*, and *MutH*) and constructed template DNA to clone the genes upstream of the SBP−protein D coding sequence (Figure 1C). Figure 5 shows typical frequency deceases (corresponding to mass increases) after the addition of EF-G for translation of mRNA encoding different proteins of interest in the presence of MRE600 and Rosetta tRNAs. The time lags obtained are summarized in Table 1 and Figure 6A.

The blue and red lines in Figure 6A show the linear relationships obtained with $(T7\ tag)_n$ model proteins in the presence of MRE600 and Rosetta tRNAs (as already shown in Figure 3). The time lags for various proteins such as CspA, IF1, NirD, HyaE, DHFR, CAT, and MutH basically increased with the length of protein translated but fell on the blue line, albeit with some deviations in the presence of MRE600 tRNAs. Translation rates (in aa $min^{-1}$) were calculated from the time lags and are shown in Figure 6B. Although translation rates for $(T7\ tag)_n$ model proteins were constant (17 ± 1 aa $min^{-1}$),


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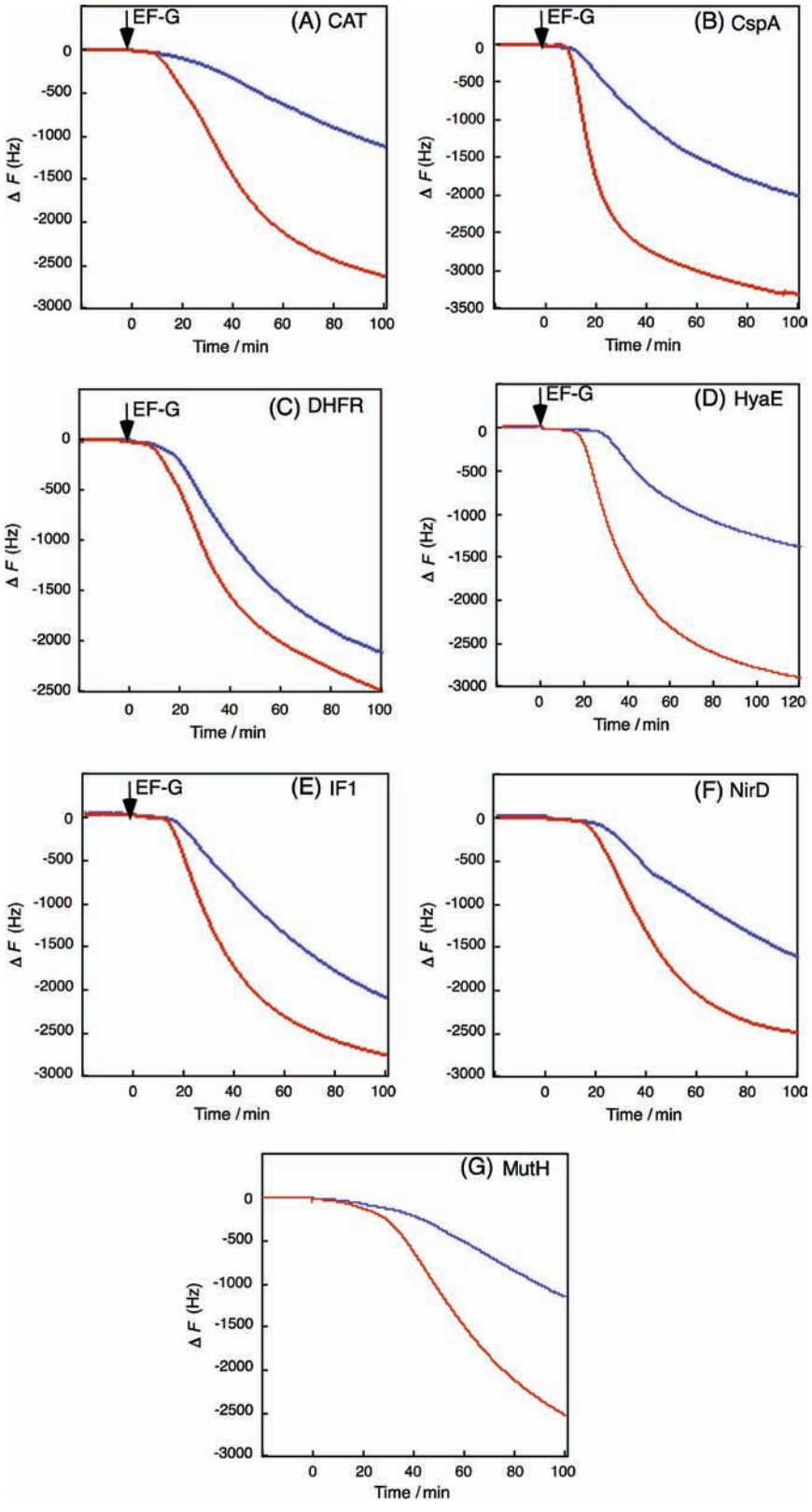


**Figure 5.** Typical frequency decreases $\Delta F$ in response to the addition of EF-G with mRNA encoding the following proteins: (A) CAT, (B) CspA, (C) DHFR, (D) HyaE, (E) IF1, (F) NirD, and (G) MutH. The blue and red curves indicate translation of $(T7\ tag)_n$ model proteins in the presence of MRE600 tRNAs and Rosetta tRNAs, respectively.

those for the various natural proteins varied from 9.3 to 20.1 aa $min^{-1}$. This clearly indicates that the traveling time of ribosomes along mRNA mainly depends on the length of the encoded protein, although rare codons may contribute to the time lag, as these proteins contained 0−3 rare codons in their sequences. In the presence of Rosetta tRNAs, however, the time lags did not consistently fall on the red line (compare the circles and squares in Figure 6A; also see Table 1), and the


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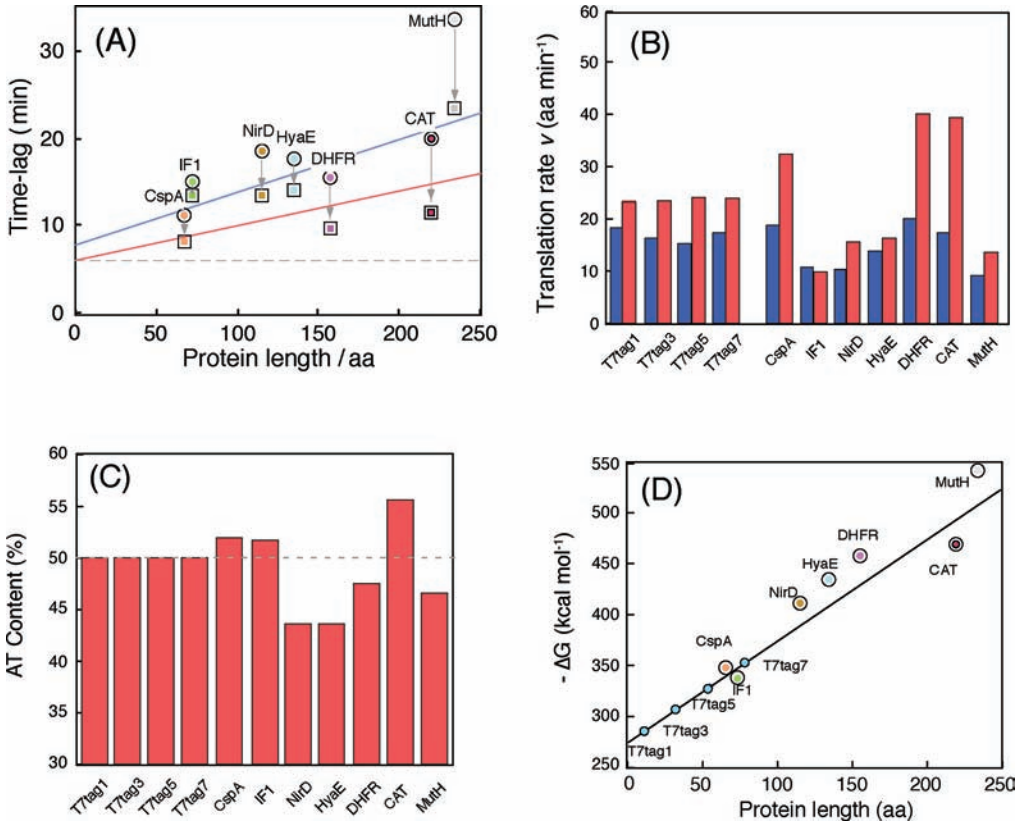


**Figure 6.** (A) Relationship between time lag and protein length obtained from mRNAs encoding various proteins of interest in the presence of MRE600 tRNAs (circles) or Rosetta tRNAs (squares). The blue and red lines show the regression lines obtained from $(T7\ tag)_n$ model proteins, as shown in Figure 3. (B) Translation rates ($v$) of mRNAs encoding various proteins of interest in the presence of MRE600 tRNAs (blue bars) and Rosetta tRNAs (red bars). The elongation rates include a 10% error in each value. (C) AT contents of mRNAs encoding various proteins. (D) Relationship between the value of $-\Delta G$ obtained from MFOLD and the protein length.

translation rates varied from 13.7 to 40.3 aa $min^{-1}$ (Figure 6B). These results suggest that the difference in the rates derives from a codon-specific factor such as the secondary structure of mRNA.

It has been suggested that high GC content and the secondary structure of the mRNA can regulate the rate of translation.[36,37] To examine the contribution of GC content to translation, we characterized the correlation between the translation rate and the AT content (Table S2 in the Supporting Information), which has the opposite meaning of GC content. As shown in Figure 6C, CspA and CAT have relatively high AT contents (low GC contents) whereas NirD, HyaE, and MutH have low AT contents (high GC contents). These tendencies corresponded well to the values of translation rate ($v$) in the presence of Rosetta tRNA (red bars in Figure 6B). However, the AT content of IF1 and DHFR did not satisfy the correlation. We also calculated the total free energies $\Delta G$ of the structured mRNAs using MFOLD[24] (the $\Delta G$ values in kcal $mol^{-1}$ are given in Table S2) and plotted them versus the protein length. As shown in Figure 6D, the $\Delta G$ values for the $(T7\ tag)_n$ model proteins showed a good linear relationship, indicating that structure stabilities did not contribute to the translation rate. As is the case with the results of AT content, NirD, HyaE, DHFR, and MutH showed relatively high structure stability, whereas IF1 and CAT showed low stability. Therefore, it is possible that the rate of translation of CspA, NirD, HyaE, CAT, and MutH depend on the secondary structure of the mRNAs, whereas IF1 and DHFR contain some structured regions where the traveling of the ribosome is locally regulated or specific sequences that interact with the ribosome like the internal SD homology. From these results, we have established that the traveling rate of a ribosome along an mRNA is determined by the effects of rare codons as much as by other effects such as RNA sequence, secondary structure of mRNA, or protein nascent chain synthesis.

## ■ CONCLUSION

In conclusion, monitoring translation on a QCM permitted us to determine the traveling time of ribosomes along various mRNA coding regions by measuring mass changes. By using a reconstituted cell-free mixture, we could easily tune the content of the reaction solution. For example, EF-G could be removed in advance to control translation initiation, and tRNAs could be exchanged for the study of rare codons. In a model experiment in which mRNAs encoding tandem repeats of T7 tags were employed, the traveling time clearly depended on the length and codon usage of the protein of interest. On the other hand, the internal SD sequence greatly affected the ribosomes translating through the region. Furthermore, the times required to translate the various native proteins were independent of each other. These results imply that the regulation of gene expression is dynamically controlled by the coding sequence itself. This is the first report to describe the use of a QCM assay to study the translation of any sequences of interest. We expect that this technique can be also used to investigate other issues concerning ribosome translation, such as aberrational stall


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during translation, cotranslational folding, and the contribution of such phenomena to diseases.

## ■ ASSOCIATED CONTENT

### Supporting Information

Tables S1 showing components of the translation mixture in the QCM cell and Table S2 showing the AT contents and $\Delta G$ values for the proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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