

Commentary

On the mechanical force generated by EF-G-catalyzed ribosome translocation

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Abstract

The determination of the amplitude of the power stroke produced by the elongation factor G-catalyzed ribosome translocation is an important issue, having strong implications for the molecular mechanism of the translocation. A recent paper (Yao *et al.*, *Angew. Chem. Int. Ed.* 2013, 52, 14041 - 14044) tried to measure the amplitude of this power stroke. We show here

that the explanation of the experimental data in the paper could be unreasonable and thus the amplitude of the power stroke determined there could be unreliable. We re-explain the experimental data and show that from these data the amplitude of the power stroke cannot be determined. Moreover, we give a different prediction of the amplitude of the power stroke.

Introduction

During translation, the ribosome reads the codons (triplets of nucleotides) on a single-stranded mRNA to synthesize the polypeptide of a protein. It has been well documented that the ribosome can also translate through the duplex region of the mRNA by unwinding the duplex (Qu *et al.* 2011, Takyar *et al.* 2005, Wen *et al.* 2008). Thus, an interesting issue is to determine the amplitude of the mechanical force provided by the ribosome in its mRNA unwinding process. In a recent paper, Yao *et al.* (2013) made an effort towards this issue. They used the binding forces of a series of RNA/DNA duplexes as internal force references to determine the amplitude of the mechanical force provided by the elongation factor (EF) G-catalyzed ribosome translocation. First, the binding forces of a series of RNA/DNA duplexes with successive numbers of complementary base pairs were determined. Then, by claiming that the mechanical force produced by the EF-G-catalyzed ribosome translocation is used to dissociate the duplexes, they used the characterized duplexes as internal force references for measuring the amplitude of the power stroke.

In this commentary, we show that the claim that the mechanical force produced by the EF-G-catalyzed ribosome translocation is used to dissociate the duplexes could be incorrect. Thus, the explanation of the experimental data by Yao *et al.* (2013) could be unreasonable and the amplitude of the power stroke determined there could be unreliable. We re-explain

the experimental data of Yao *et al.* (2013) and show that from their experimental data the amplitude of the power stroke cannot be determined. Moreover, we calculate the amplitude of the mechanical force that can be generated by the ribosome, which can be tested by future experiments.

Explanation of experimental data of Yao *et al.* (2013)

Using the nearest-neighboring (NN) thermodynamic model for the stability of RNA/DNA hybrids (Wu *et al.*

Table 1. The binding energy of the mRNA strand with the DNA strand used in Yao *et al.* (2013), which are calculated using the NN-model free energy parameters at 37 °C for RNA/DNA hybrids (Wu *et al.* 2002)

	No ribosome	POST
Number of bps	Binding energy ($k_B T$)	Binding energy ($k_B T$)
10 bp	10.65	5.92
13 bp	17.91	11.49
14 bp	20.28	13.96
15 bp	20.96	14.53
16 bp	23.66	17.24
17 bp	25.18	18.76
18 bp	26.53	20.11
13 bp shifted	20.62	17.24



Figure 1. The sequences of the DNAs and mRNA used in Yao *et al.* (2013). The green oval on the mRNA indicates the portion covered by the 30S subunit of the ribosome in PRE state, with the 30S A, P and E sites being indicated. The yellow oval on the mRNA indicates the portion covered by the 30S subunit of the ribosome in POST state. To be consistent with the available experimental data (Qu *et al.* 2011, Takyar *et al.* 2005), we draw here that the ribosome covers 12 bases of the single-stranded mRNA from its P site to the mRNA entry site. The bases in red on the DNAs highlight the complementary bases to the mRNA.

al. 2002), we calculate the binding energy of the mRNA strand to the DNA strand used in Yao *et al.* (2013) (see Figure 1). In the absence of the ribosome, the results are shown in Table 1 (no ribosome). In the presence of the ribosome in the pretranslocation (PRE) state with deacylated tRNA^{Met} in the 30S P site and peptidyl-tRNA^{Phe} in the 30S A site, the region of the mRNA covered by the 30S subunit of the ribosome is shown by green oval in Figure 1. As the ribosome covers about 12 bases of the single-stranded mRNA from its P site, which is now occupying codon AUG (blue), to the mRNA entry site (Qu *et al.* 2011, Takyar *et al.* 2005), the ribosome should unwind three A/T base pairs in the 10 to 18 base pair (bp) RNA/DNA hybrids. After the binding of EF-G guanosine triphosphate (GTP), the pretranslocation (PRE) state is transitioned to the posttranslocation (POST) state via a downstream movement of the ribosome by one codon, while the region of the mRNA covered by the 30S subunit of the ribosome is depicted as the yellow oval in Figure 1. Since it has been well documented that the ribosome can unwind mRNA duplex during translation (Qu *et al.* 2011, Takyar *et al.* 2005, Wen *et al.* 2008), it is taken for granted here that the translocation should induce the unwinding of three RNA/DNA (i.e., UCU/AGA) base pairs. Thus, in the posttranslocation (POST) state, only the duplex region of the RNA/DNA hybrids in the left-hand side of the green line remains unwound. The calculated results for the binding energy of the remaining region of the mRNA strand to the DNA strand in the POST state are shown in Table 1 (POST).

From Table 1, it can be seen that in the POST state the binding energy of the mRNA with the DNA strand for the 17 bp hybrid is $\Delta G_{POST}^{(17bp)} = 18.76k_B T$,

which is between the binding energy for the 13 bp hybrid in the absence of the ribosome, $\Delta G_{No}^{(13bp)} = 17.91k_B T$, and for the 14 bp hybrid, $\Delta G_{No}^{(14bp)} = 20.28k_B T$. As the experimental data of Yao *et al.* (2013) showed, the binding forces for the 13 bp and 14 bp hybrids in the absence of the ribosome are about 36 and 52 pN, respectively; it is thus expected that the binding force for the 17 bp hybrid in the POST state should be larger than 36 pN and smaller than 52 pN. This is consistent with the data of Yao *et al.* (2013) showing that the binding force for the 17 bp hybrid in the POST state is about 50 pN.

The mean dissociation time of the mRNA strand from the fixed DNA strand can be calculated by

$$(1) \quad \tau = C \exp\left(-\frac{\Delta G}{k_B T}\right)$$

where C is a constant and ΔG is the binding energy of the mRNA with the DNA, as shown in Table 1. Similar to the procedure used before (Xie 2013a), one can easily derive that the distribution of the dissociation time of the mRNA from the DNA has the form

$f(t) = \tau^{-1} \exp(-t/\tau)$. The remnant fraction of the mRNAs that have not been dissociated from the DNA strands after transition to the POST state by time T_0 is calculated by $P_R = \int_{T_0}^{\infty} f(t) dt$, which can be rewritten as

$$(2) \quad P_R = \exp\left(-\frac{T_0}{\tau}\right)$$

With values of ΔG given in Table 1 (POST), using Eqs. (1) and (2) we calculate the remnant fraction

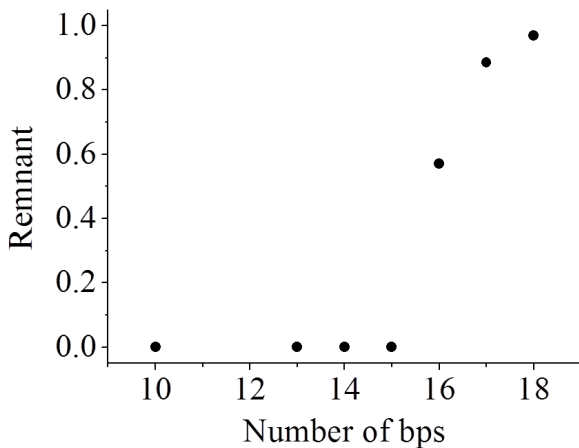


Figure 2. Calculated results of the remnant fraction of the RNA/DNA duplexes after the ribosomal translocation versus the number of complementary base pairs. Here we adjust C and T_0 to make the remnant fraction P_R for the 16 bp duplex to be consistent with the experimental data.

P_R , with the results shown in Figure 2. It can be seen that the results of this figure are consistent with the experimental data (Figure 4 in Yao *et al.* (2013)).

Thus, we conclude that the mechanical force generated by the ribosome during translocation is used here to unwind the three UCU/AGA base pairs of the RNA/DNA hybrid rather than to dissociate the whole RNA/DNA hybrid, as suggested by Yao *et al.* (2013). The dissociation of the RNA strand from the DNA strand after the translocation is due to the fact that the number of base pairs in the hybrid is reduced, resulting in a great decrease in the binding energy of the RNA with the DNA strand. This implies that the mechanical force generated by the ribosome during translocation should not be determined by using the binding forces of a series of RNA/DNA duplexes as internal force references. Consequently, the amplitude of the power stroke of 89 ± 9 pN determined in Yao *et al.* (2013) could be unreliable.

The amplitude of the power stroke by the ribosome

In this section, we provide a prediction of the amplitude of the power stroke that can be generated by the ribosome.

Model of ribosomal translocation

Available experimental data have showed that the ribosomal unlocking occurring in the hybrid state bound with EF-G.GDP.Pi opens the mRNA channel, greatly reducing the interaction between the mRNA-tRNA complex and the 30S subunit (Savelsbergh *et al.* 2003). The 50S E site has a high affinity for deacylated tRNA and the 50S P site has a specific affinity for the peptidyl moiety (Feinberg & Joseph 2001, Lill *et al.* 1989). Based on the above lines of experimental evi-

dence, we have previously proposed a model of the ribosome translocation (Figure 3) (Xie 2013b, 2014). After the ribosomal unlocking in the hybrid state (Figure 3C), if no external force is present to resist the forward movement of the 30S subunit along the mRNA, the reverse rotation of the 30S subunit relative to the 50S subunit would induce the two tRNAs coupled with the mRNA to move from the 30S P and A sites to the 30S E and P sites, while the two tRNAs are kept fixed to the 50S E and P sites by their large affinity for the two tRNAs (Figure 3D). The transition from Figure 3C to 3D induces an effective translocation. If a very large external force is present to resist the forward movement of the 30S subunit along the mRNA, which prevents the mRNA that is coupled with the two tRNAs from moving forward relative to the 30S subunit, the reverse rotation of the 50S subunit relative to the 30S subunit would induce the two tRNAs to move from the 50S E and P sites to the 50S P and A sites by overcoming the large affinity of the two tRNAs for the 50S E and P sites (Figure 3E). The transition from Fig. 3C to 3E induces no translocation, which is called futile translocation. For an intermediate resistant force, the reverse intersubunit rotation would induce the transition of the hybrid state of Fig. 3C either to the state of Fig. 3D (the effective translocation) or to the state of Fig. 3E (the futile translocation). As proposed by Frank & Agrawal (2000), after transition to the non-rotated conformation (Figure 3D or 3E), the mRNA channel becomes tight again, with the interaction between the mRNA-tRNA complex and the 30S subunit becoming very strong.

As shown before (Xie 2013b, 2013c, 2014), considering the futile translocation, the intriguing single-molecule optical trapping data of Qu *et al.* (2011) showing a sigmoid dependence of the translation rate through the mRNA duplex on the external force to unzip the duplex can be quantitatively explained (Xie 2013b); the smFRET data of Chen *et al.* (2013a) showing that the downstream mRNA secondary structures have a more sensitive effect on deacylated tRNA dissociation than on tRNA translocation in the 50S subunit can also be explained well (Xie 2014). Moreover, the intriguing experimental evidence showing that although the occurrence of the -1 frameshifting (FS) at the slippery sequence is usually associated with the occurrence of the ribosomal pausing, the pausing alone being insufficient to promote FS (Kontos *et al.* 2001, Lopinski *et al.* 2000, Somogyi *et al.* 1999, Tu *et al.* 1992), can also be explained well (Xie 2013c).

Calculation of the amplitude of the power stroke

Here, based on the model (Figure 3) we calculate the amplitude of the mechanical force that can be generated by the ribosome during its translocation, which corresponds to the stall force of the ribosome. Consider an external force F resisting the forward translocation of the 30S subunit along the single-stranded mRNA. After the ribosomal unlocking, which

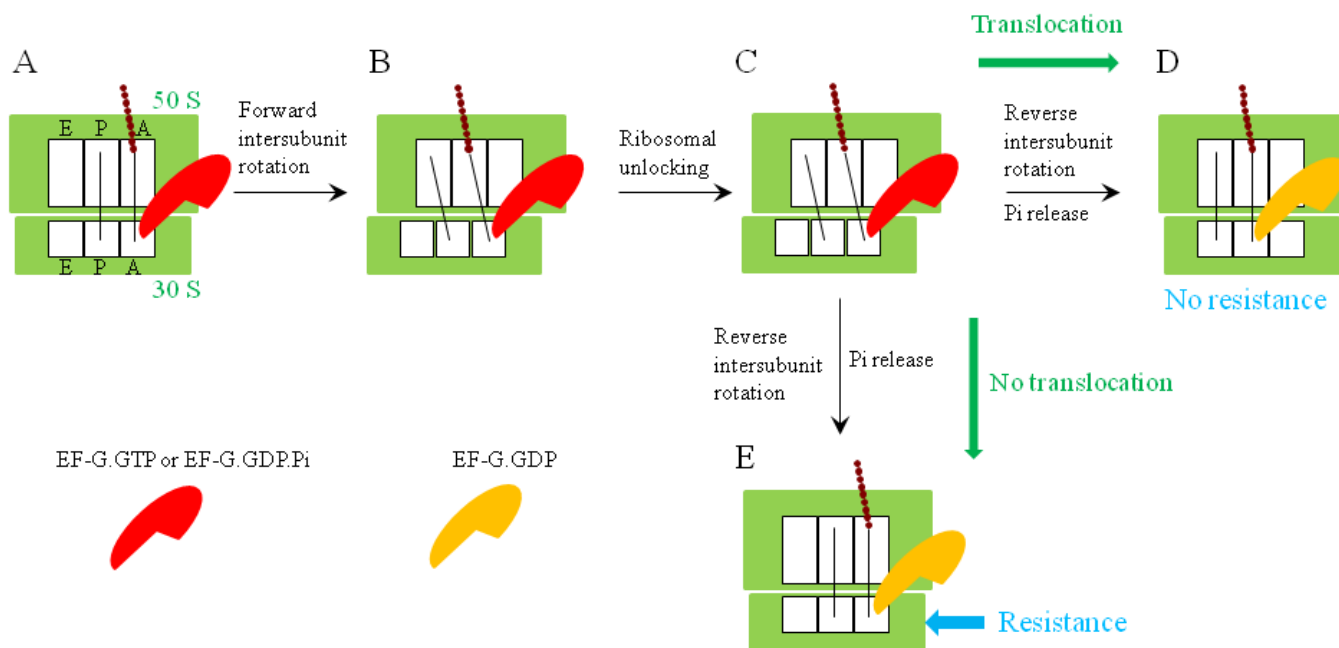


Figure 3. Schematic representation of the model of the ribosome translocation catalyzed by EF-G in the presence of GTP. After EF-G.GTP binding to the classical non-rotated PRE state (A), GTP hydrolysis occurs rapidly prior to forward intersubunit ribosomal rotation (B). In the hybrid state bound with EF-G.GDP.Pi, the ribosomal unlocking occurs (C). If no external force is present to resist the forward movement of the 30S subunit, the reverse intersubunit rotation induces effective translocation (D). If a very large external force is present to resist the forward movement of the 30S subunit, the reverse intersubunit rotation induces futile translocation (E). For an intermediate resistant force, the reverse intersubunit rotation would induce either the effective translocation (C) or the futile translocation (E).

greatly reduces the interaction between the mRNA-tRNA complex and the 30S subunit, whether the reverse intersubunit rotation induces the effective translocation or induces the futile translocation is determined by the competition between the binding energy of the 50S E and P sites for the two tRNAs and the work that can be done by the resistant force F during the rotation of the 30S subunit relative to the 50S subunit. The probability of the forward translocation of the 30S subunit induced by the reverse intersubunit rotation can be calculated by

$$(3) \quad \varepsilon = \frac{\exp(-Fd/k_B T)}{\exp(-Fd/k_B T) + \exp(E_{PE}^{(50S)}/k_B T)}$$

where $d = 3p$, with $p = 0.34$ nm being the distance between two successive bases on the single-stranded mRNA, and $E_{PE}^{(50S)}$ is the difference between the high binding energy of the 50S E and P sites for the two tRNAs and the low binding energy of the 30S subunit for the mRNA-tRNA complex after the ribosomal unlocking.

It is noted that both the POST state of Fig. 3D and the PRE state of Figure 3E are bound with EF-G.GDP. As the single-molecule data showed that the lifetime of EF-G.GDP bound to the PRE state is much longer than that bound to the POST state (Chen *et al.* 2013b), it is

deduced that the rate of EF-G.GDP release from the PRE state (Figure 3E) is much smaller than that from the POST state (Figure 3D). With the approximation that the time of EF-G.GDP release from the PRE state of Figure 3E is comparable to that from the POST state of Figure 3D plus the time from the binding of the aminoacyl-tRNA.EF-Tu.GTP ternary complex at saturating concentration through the peptidyl transfer, as done before (Xie 2013b), the dwell time of the ribosome translation induced by the resistant force F is calculated by $T_{dwell} = (\varepsilon v)^{-1}$. With Eq. (3), T_{dwell} has the form

$$(4) \quad T_{dwell} = \frac{\exp(-Fd/k_B T) + \exp(E_{PE}^{(50S)}/k_B T)}{v \exp(-Fd/k_B T)}$$

where v is the mean translation rate through the single-stranded mRNA, without the external force F to resist the forward ribosome translocation.

As shown before (Xie 2013b), the fitted value of $E_{PE}^{(50S)}$ is about $9k_B T$. Previous experimental data have shown that v is in the order of 1 S^{-1} (Wen *et al.* 2008). With $E_{PE}^{(50S)} = 9k_B T$ and $v = 1 \text{ S}^{-1}$, using Eq. (4) we calculate the mean dwell time T_{dwell} versus the resistant force F , with the results shown in Figure

4. As expected, the longer the dwell time is, the larger the resistant force required is. A large force is required to make the ribosome be stalled for a long time. For example, about a 53 pN force is required to make the ribosome be stalled for 1 min and about a 62 pN force is required to make the ribosome be stalled for 10 min. These results imply that the ribosome can generate a mechanical force with a large magnitude that is close to that determined in Yao *et al.* (2013), although their determination method could not be correct.

Conclusions

By analyzing the EF-G.GTP-catalyzed ribosome translocation through the single-stranded mRNA with the downstream region forming base pairs with the DNA strand, we show that the translocation-induced dissociation of the mRNA from the fixed DNA strand observed in Yao *et al.* (2013) is due to the reduction of the number of base pairs in the hybrid, i.e., the great decrease in the binding energy of the RNA with the DNA strand, rather than due to the EF-G power stroke acting on the DNA strand. Thus, the amplitude of the power stroke obtained by using the binding forces of a series of RNA/DNA duplexes as internal force references could be unreliable. Moreover, based on our previously proposed model, we provide a prediction of the amplitude of the power stroke that can be generated by the ribosome, with an approximately 53 pN force being required to make the ribosome be stalled for 1 min and a 62 pN force being required to make the ribosome be stalled for 10 min.

Conflicts of interest

The author has no conflicts of interest.

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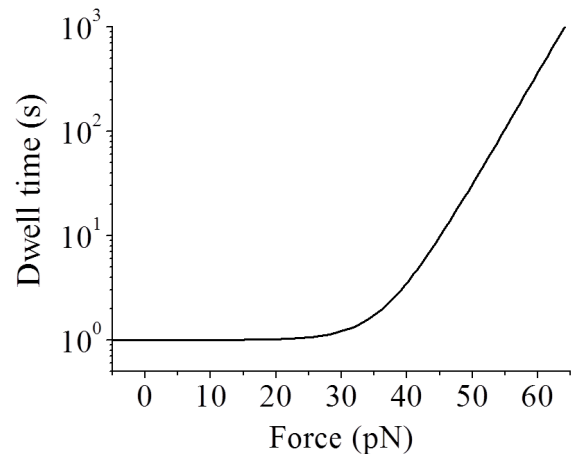


Figure 4. Calculated results of the mean dwell time versus the external force resisting the forward translocation of the 30S subunit along the single-stranded mRNA.

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