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Model of the pathway of –1 frameshifting: Kinetics

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ABSTRACT

Programmed –1 translational frameshifting is a process where the translating ribosome shifts the reading frame, which is directed by at least two stimulatory elements in the mRNA—a slippery sequence and a downstream secondary structure. Despite a lot of theoretical and experimental studies, the detailed pathway and mechanism of the –1 frameshifting remain unclear. Here, in order to understand the pathway and mechanism we consider two models to study the kinetics of the –1 frameshifting, providing quantitative explanations of the recent biochemical data of Caliskan et al. (Cell 2014, 157, 1619–1631). One model is modified from that proposed by Caliskan et al. and the other is modified from that proposed in the previous work to explain the single-molecule experimental data. It is shown that by adjusting values of some fundamental parameters both models can give quantitative explanations of the biochemical data of Caliskan et al. on the kinetics of EF-G binding and dissociation and on the kinetics of movement of tRNAs inside the ribosome. However, for the former model some adjusted parameter values deviate significantly from those determined from the available single-molecule experiments, while for the latter model all parameter values are consistent with the available biochemical and single-molecule experimental data. Thus, the latter model most likely reflects the pathway and mechanism of the –1 frameshifting.

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1. Introduction

Programmed ribosomal frameshifting is a process where specific signals in the messenger RNA (mRNA) direct the translating ribosome to shift the reading frame. When the reading frame is shifted in the 3′ direction or the 5′ direction by one nucleotide, it is called +1 or −1 frameshifting, respectively. The classic example of the −1 frameshifting contains two stimulatory signals in the mRNA—a slippery sequence and a downstream secondary structure [1–3]. In some prokaryotic cases such as the *dnaX* –1 frameshifting mRNA, three stimulatory signals—an upstream, internal Shine-Dalgarno (SD) sequence, the slippery sequence and the downstream mRNA duplex —are necessary to stimulate the efficient −1 frameshifting [3].

Recently, using single-molecule fluorescence to track directly the compositional and conformational dynamics of individual ribosomes at each codon, Chen et al. [4] studied the −1 frameshifting during translation of the *dnaX* –1 frameshift mRNA. The dynamics of long pausing associated with the −1 frameshifting and the dynamics of EF-G and tRNA samplings in the long-paused state were studied in detail [4]. Using single-molecule fluorescence resonance energy transfer (FRET), Kim et al. [5] found that the pretranslocation ribosomal complexes exhibit multiple fluctuations between the non-rotated and rotated states before undergoing mRNA translocation during translation of the *dnaX* –1 frameshift mRNA even at saturating EF-G. With the PURExpress in vitro translation system, Yan et al. [6] found that the ribosomes can undergo several translocation excursions to shift reading frame and access a range of codon positions. Caliskan et al. [7] made detailed biochemical studies on the kinetics of the translocation reactions that govern the –1 frameshifting in the system with a modified IBV 1a/1b gene fragment (see Fig. S1a). They studied the kinetics of EF-G binding and dissociation by monitoring the change in the fluorescence resonance energy transfer (FRET) between a FRET donor (Alexa 488, Alx) placed on ribosomal protein L12 that is known to recruit translation factors to the ribosome and a non-fluorescent FRET acceptor (QSY9) in the G domain of EF-G, and studied the kinetics of movement of tRNAs inside the ribosome by monitoring the change in the fluorescence of fluorescein (Flu) labeled at $tRNA^{Leu}$ and the change in FRET between S13(AttoQ) of the 30S subunit and the $tRNA^{Leu}$(Flu). Based on their biochemical data, they proposed a model for the pathway of the –1 frameshifting (see Fig. S1). However, a quantitative study based on the model indicates that the calculated results are not consistent with the biochemical data (see Section S1). Thus, to understand the pathway and mechanism of the –1 frameshifting, a modified model or a new model that can provide quantitative explanations of the biochemical data [7] is necessary.

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Several models have been proposed to address the −1 frameshifting pathway and mechanism. It was proposed that the −1 frameshifting can occur at the aminoacyl-tRNA accommodation step [8,9], at the translocation step [10,11], or at both of the two steps [12]. A systematical analysis proposed that while the −1 frameshifting can occur during the translocation step, during the period after the posttranslocation and before the binding of the aminoacyl-tRNA and during the period after the codon recognition and before the peptidyl transfer, the frameshifting takes place mainly during the translocation over the slippery sequence [13]. In the previous work [14], a new model of the −1 frameshifting was proposed to quantitatively explain the recent single-molecule experimental data of Chen et al. [4] on the dynamics of long pausing that is associated with the −1 frameshifting. The single-molecule FRET data of Kim et al. [5] and the experimental data of Yan et al. [6] were also explained [14,15]. In the models [8–14] only the intersubunit rotations between the 50S and 30S subunits are considered. However, structural and biochemical studies showed that besides the intersubunit rotations, the intrasubunit rotations of the 30S head relative to the 30S body are also involved in the translocation of the tRNA-mRNA complex in the 30S subunit [16–20]. As the −1 frameshifting occurs mainly during the translocation step, it is necessary to incorporate the intrasubunit 30S head rotations in the model of the −1 frameshifting. Moreover, with the previous model by considering only the intersubunit rotations, although the experimental data of Chen et al. [4], Kim et al. [5] and Yan et al. [6] can be explained well [14], the biochemical data of Caliskan et al. [7] on the kinetics of the −1 frameshifting, which are monitored by labeling the ribosomal protein of the 30S head, cannot be explained.

In this work, to understand the detailed molecular mechanism and pathway of the −1 frameshifting and to quantitatively explain the biochemical data of Caliskan et al. [7], we consider two models, with one being modified from the model proposed by Caliskan et al. [7] and another one being modified from the previous model [14] by also considering the intrasubunit 30S head rotations. It is shown that by adjusting values of some fundamental parameters both modified models can give quantitative explanations of the biochemical data of Caliskan et al. [7]. However, for the former model some adjusted parameter values deviate significantly from those determined from the single-molecule experiments of Chen et al. [4], while for the latter model all of the adjusted parameter values are consistent with the available biochemical and single-molecule experimental data. Thus, we believe that the latter model most likely reflects the pathway and mechanism of the −1 frameshifting.

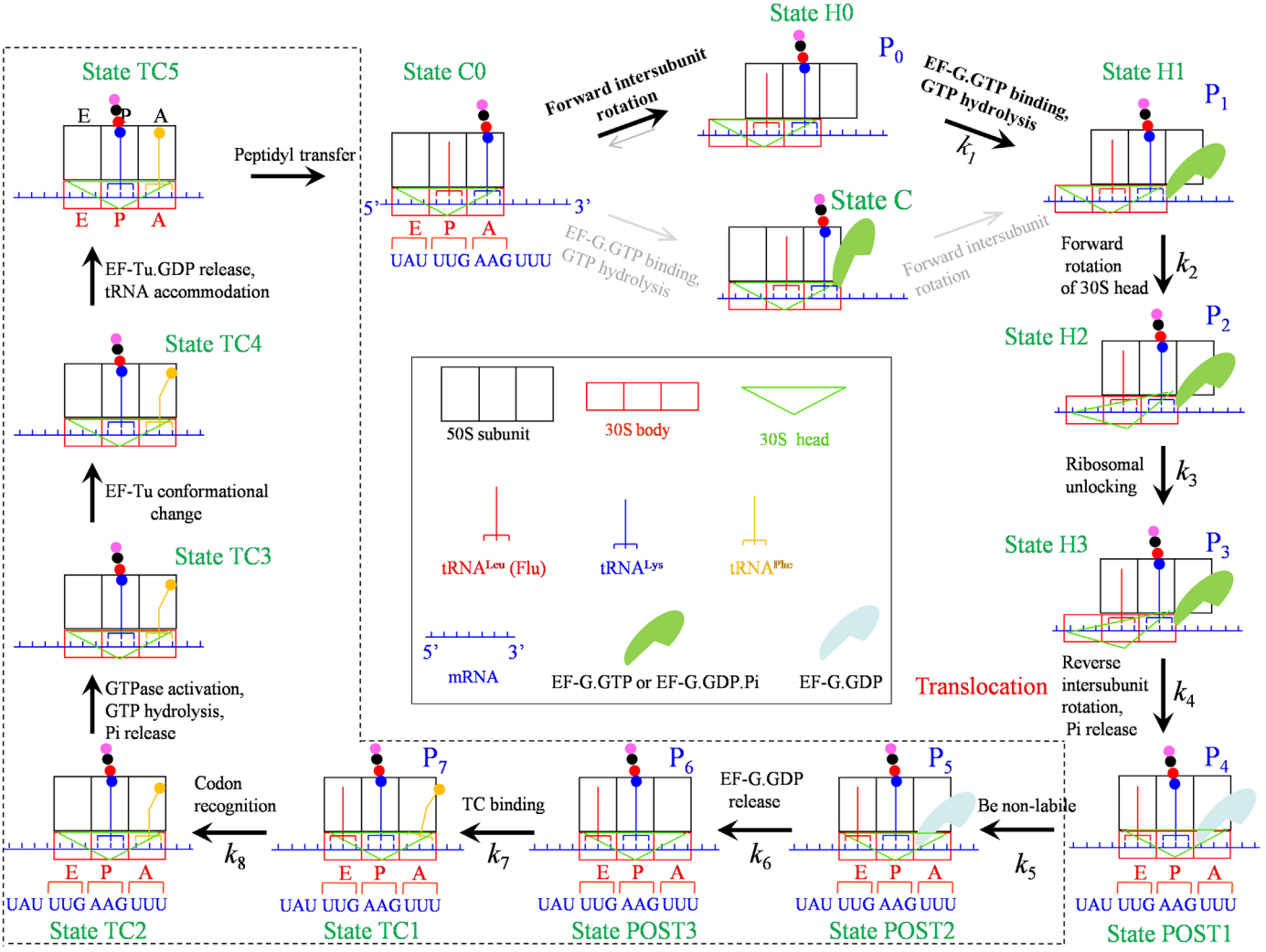

Fig. 1. Schematic representation of the elongation pathway for ribosome translation of mRNA lacking both the slippery sequence and downstream secondary structure (see text for detailed description). Note that EF-G bound to the non-rotated pretranslocation state (State C) is in the compact conformation while EF-G bound to the rotated pretranslocation state (State H1, State H2, State H3) and bound to the non-rotated posttranslocation state (State POST1, State POST2) is in the elongated conformation [28].

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