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Dynamics of backtracking long pauses of RNA polymerase

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ABSTRACT

The dynamics of backtracking long pauses by RNA polymerase is quantitatively studied based on our proposed model. Analytical formulas are obtained for the dependence of mean backtracking time and mean lifetime of backtracking long pauses on the binding affinity V_0 of RNAP to the DNA and RNA lattices, an important parameter for the transcription elongation complex. By comparison of the theoretically with experimentally obtained mean backtracking times, the value of binding affinity V_0 is predicted. Using the predicted V_0, the effects of external load on the mean backtracking time, on the lifetime of the backtracking long pauses and on the exit from the backtracking long pauses are studied. The results are in agreement with the available experimental data and, moreover, some predicted results are presented. In addition, using this V_0 we study the processivity of RNA polymerase under no and sideways forces.

1. Introduction

RNA polymerase (RNAP) is an enzyme that catalyzes the synthesis of RNA on a DNA template, the first stage in the overall process of gene expression [1,2]. A peculiar feature of RNAP is that the rate at which it adds nucleotide to 3′ end of the growing RNA transcript is highly nonuniform. At some sites along the template, RNAP falls into "off-pathway" states, displaying various elongation pauses [2–8].

Over the past decade a great deal has been learned about the structures of RNAP [10–17]. Meanwhile, much progress has been made in elucidating the mechanism of elongation transcription and in studying the kinetic and dynamic behaviors such as the moving velocities and pausing characteristics by using biochemical assays and single-molecule techniques [18–27]. *In vitro* experimental studies with *Escherichia coli* RNAP have classified the elongation pauses into long (>20 s) and short (1–6 s) pauses [23–25]. It has been shown that, over all pauses, about 95% are short pauses while remaining 5% are long pauses [24]. The short pauses have been shown to be triggered by common sequence signals rather than random events [25]. The long pauses were usually linked with the reverse translocation of RNAP (backtracking), which may be caused by nucleotide misincorporation or a weak RNA–DNA hybrid [9,24,28]. In contrast to the short pauses, the lifetime and occurrence frequency of which are insensitive to external loads [24], both the lifetime and occurrence frequency of the backtracking long pauses are sensitively dependent on the external loads [24].

Theoretically, several models have been presented for the mechanism of transcription elongation by RNAP and these models can broadly fall into two classes. One class, called the power stroke mechanism, proposes that the conformational change (i.e., the rotation of the O helix from the closed to semi-open conformations) in the single-subunit T7 RNAP tightly coupled to PPi release drives the motion [29]. By comparative analysis of the two F bridge conformations in multi-subunit bacterial and yeast RNAP structures, Kornberg and coworkers hypothesized that the change of F bridge helix from the straight to the bent conformation induces translocation of nucleic acids while the reversion to the straight conformation without movement opens the substrate binding site for the next complementary NTP [13]. Another class, called the Brownian ratchet mechanism, postulates that thermal energy in the form of Brownian motion imparts the kinetic energy to the RNAP and that the motion is biased in one direction by the binding of the incoming NTP [18,26,30]. Pioneered by the works of von Hippel and colleagues [31,32], the kinetics of transcription elongation and pausing has been extensively studied by using a static sequence-dependent thermodynamic analysis of transcription elongation complex (TEC) stability [30,33–35]. In particular, the quantitative predictions of transcription pausing have been presented [36,37]. By assuming a symmetric hopping process with a constant hopping rate, Voliotis et al. [38] have presented a quantitative study of the dynamics of backtracking pauses.

In the previous work [39], we have presented a new thermal ratchet model for the transcription elongation by RNAP, where the forward translocation of RNAP along the DNA template is directionally rectified through the incorporation of a matched base. The sequence-dependent short pauses, which are assumed to be resulted mainly from the site-specific strong bindings of RNAP to dsDNA and/or RNA–DNA hybrid, have been quantitatively studied, with the results being in good agreement with the available experimental data.

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In this work, based on our proposed model, we quantitatively study the dynamics of the backtracking long pauses by using the relevant parameter values inferred from the available experimental data. By comparison of the theoretically with experimentally obtained mean backtracking times, the value of an important parameter for TEC, i.e., the binding affinity, V_0, of RNAP for the DNA and RNA lattices, is predicted. Furthermore, using this predicted V_0, we study the effects of external load on the mean backtracking time, on the lifetime of the backtracking long pauses and on the exit from the backtracking long pauses. To test the validity of the predicted value of V_0, we calculate the mean run length by using this V_0, with the calculated result being consistent with the experimental data. Moreover, the effect of sideways force on the processivity is studied.

2. Model of transcription elongation and backtracking long pauses

The two hypotheses in our proposed Brownian ratchet model for transcription elongation by a multi-subunit RNAP, with the supporting experimental evidences as discussed in detail in the previous paper [39], are stated as follows. (i) It is assumed that there exists an ssDNA-binding site (P) in the vicinity of the polymerase site that has a high affinity for 5′–3′ ssDNA. There also exist other binding sites that have high affinities for dsDNA and/or RNA–DNA hybrid in the RNAP. (ii) The nucleotide binding to the entrance site and then transition to the insertion site induce the stabilization of the bent F-bridge conformation, while the free nucleotide (i.e., the release of nucleotide) induces the stabilization of straight conformation. The contact of the bent F bridge helix with the nucleic acids promotes the chemical reaction of nucleotide incorporation, while the reversion to the straight F bridge helix opens the substrate binding site for next NTP binding. It is noted that the bending of F bridge helix is assumed to only make it contact with the 3′ face of the RNA–DNA hybrid, thus promoting the reaction of nucleotide incorporation, rather than to induce translocation of the nucleic acid by one nucleotide as proposed before [12–14]. Based on these two hypotheses, the transcription elongation by multi-subunit RNAP is schematically described in Fig. 1.

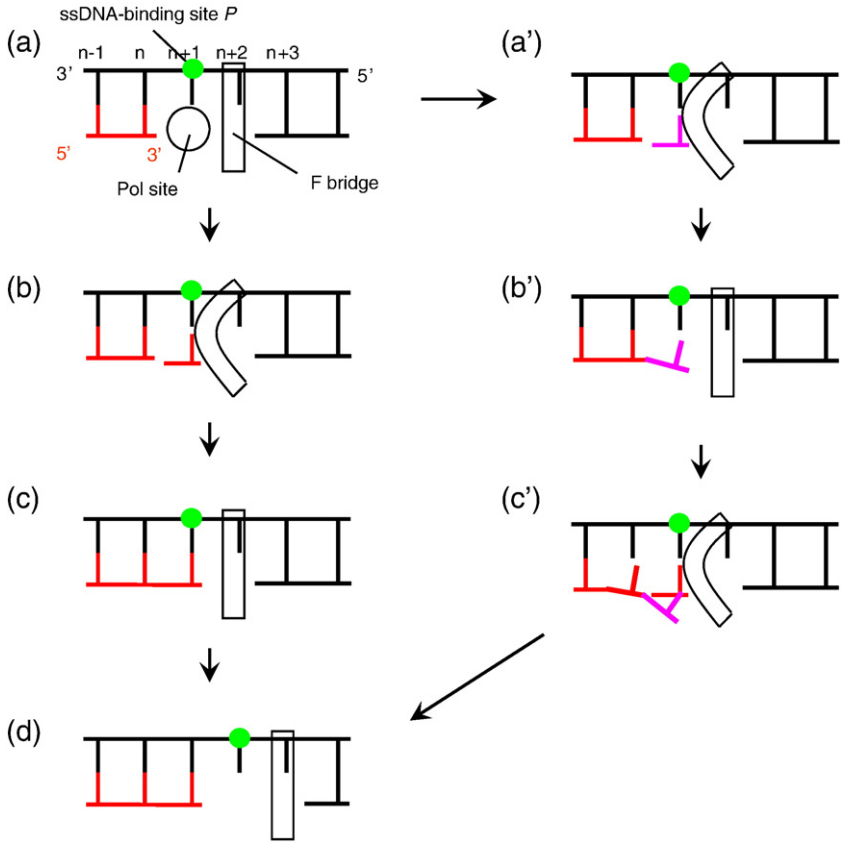

Fig. 1. Schematic illustrations of transcription elongation by multi-subunit RNAP. DNA is drawn in black and RNA in red. (a) The ssDNA-binding site P binds $(n+1)$-th base on the 5′–3′ ssDNA template near the nascent RNA–DNA hybrid. No nucleotide is bound to the polymerase active site (Pol site). Here, Pol site represents the overlapping entrance and insertion NTP-binding sites. (b) A matched NTP first binds to the entrance site and then transits to the insertion site, which induces the stabilization of the bent F-bridge conformation. The bent F-bridge conformation triggers nucleotide incorporation. (c) After the completion of nucleotide incorporation and the release of PPi, the F bridge helix is induced to stabilize in the straight conformation. (d) Concomitant with the process in (c), the ssDNA-binding site P binds the new nearest unpaired $(n+2)$-th base of the ssDNA template because the previous $(n+1)$-th base where the ssDNA-binding site P has just bound has disappeared due to base pairing. The movement from the $(n+1)$-th site to $(n+2)$-th site is driven by both the binding force from the new nearest unpaired $(n+2)$-th base and the thermal noise by overcoming the binding force to duplex DNA and/or RNA–DNA hybrid. The straight F-bridge conformation opens the substrate binding site for next NTP binding. From (a), through (b) and (c), to (d) a chemomechanical cycle is completed. (a′) A mismatched NTP binds to the entrance site and then transits, although with a low probability, to the insertion site, which induces the stabilization of the bent F-bridge conformation. For clarity, the mismatched NTP is drawn in pink. (b′) After the completion of nucleotide incorporation and the release of PPi, the F bridge helix is induced to stabilize in the straight conformation. In this case of misincorporation, although the sugar-phosphate backbone of the mismatched NTP is connected to the backbones of the nascent RNA, the mismatched base is not paired with the sterically corresponding base on the ssDNA template. Thus the ssDNA-binding site P is still binding the same unpaired $(n+1)$-th base of the ssDNA template. (c′) Another NTP binds to the nucleotide-free polymerase site and then transits to the activated substrate state. This induces the stabilization of the bent F-bridge conformation, which can trigger nucleotide incorporation. However, because of the steric obstacle from the previous incorporated sugar-phosphate backbone, the new NTP cannot be connected to the backbones of the nascent RNA and, thus, the incorporation cannot be completed. Therefore, the transcription elongation becomes stalled. Note that, if the mismatched base is excised, the new NTP becomes able to be incorporated and, after the release of PPi, the F bridge helix is then induced to stabilize in the straight conformation of (d). From (d) the next catalytic cycle will begin and thus the transcription elongation will proceed continuously again.