COMMENTARY

Unveiling translocation intermediates of RNA polymerase

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Translocation of RNA polymerase (RNAP) is a robust target for regulation of gene expression in prokaryotes and eukaryotes (1–3). During elongation, RNAP frequently encounters a broad range of DNA/RNA conformations (RNA hairpins, curved and cruciform DNA), DNA-binding proteins, DNA lesions, and misincorporation events at the 3′ ends of the RNA. These encounters impede forward translocation, leading to RNAP pausing (3). There are many protein factors that strengthen or weaken pausing by targeting translocation, such as archaeal/eukaryotic Spt5 and bacterial NusG/RfaH (4) or N/Nun proteins of lambdoid phages (3). In metazoa, RNAP II pausing in promoter-proximal regions plays a role in having polymerases in place for a rapid transcription response to environmental stimuli, such as heat-shock or cell differentiation, and maintaining a basal level of gene expression (5, 6). Translocation blocks also initiate RNAP backtracking (7). Backtracking events disengage the 3′ RNA end from the RNAP catalytic site, which stabilizes pausing; this type of event broadly controls gene transcription in eukaryotes (8). Forward translocation of RNAP along DNA has long been regarded as the movement of the RNA–DNA hybrid through the catalytic cleft, which vacates the active center, termed *i*+1 site, for an NTP binding. In this sense, the mechanism of translocation has been primarily focused on the hybrid movement, with only limited emphasis on the DNA sequences surrounding the hybrid (1, 2, 9). However, several reports indicate that the DNA sequences immediately upstream and downstream from the hybrid regulate translocation (7, 10–12). In PNAS, Silva et al. (13) identify an important component of the translocation mechanism using millisecond molecular dynamics (MD) simulation of translocation of yeast RNAP II: Translocation of the hybrid occurs before entry of the template DNA base to the *i*+1 site, where it can pair with an NTP. A similar scenario had been suggested based on the X-ray structure of RNAP II with the transcription inhibitor α-amanitin (9). The MD simulation posits that the entering of the template DNA base requires its stacking interaction with Tyr836 in the middle of the bridge helix (BH), a long α-helix separating the *i*+1 site from the downstream DNA (Fig. 1*A*). The Tyr residue in the BH is highly conserved from *Escherichia coli* to human. The bent and straight forms of the BH have been previously observed in the crystal structure of bacterial RNAP and eukaryotic RNAP II (14, 15), leading to one hypothesis that the bent-stretch transition or oscillation of the BH coupled with the movement of the trigger loop, another flexible part of the *i*+1 site involved in catalysis and substrate binding (16), is a driving force for forward translocation by forming a "pawl" (17). By revealing long-time translocation dynamics with atomic resolution, Silva et al. (13) show that the stacking interaction of the *i*+1 DNA base with the tyrosine of the BH may form an additional pawl.

Based on kinetic modeling, Silva et al. argue that the template DNA base–Tyr836 stacking generates a metastable intermediate, which decreases the high activation energy of the transition state for forward translocation (13). The MD simulation using in silico Y836V mutant lacking the aromatic side chain of tyrosine showed interrupted translocation, supporting this hypothesis. Although this argument is conceivable, it remains uncertain whether each translocation intermediate in all sequence contexts has a homogeneous state that is separated by the activation energy or the energy barrier >> 0.5 k_BT, the averaged value of thermal energy per degree of freedom, where k_B is the Boltzmann constant and T is the absolute temperature. This assumption is required

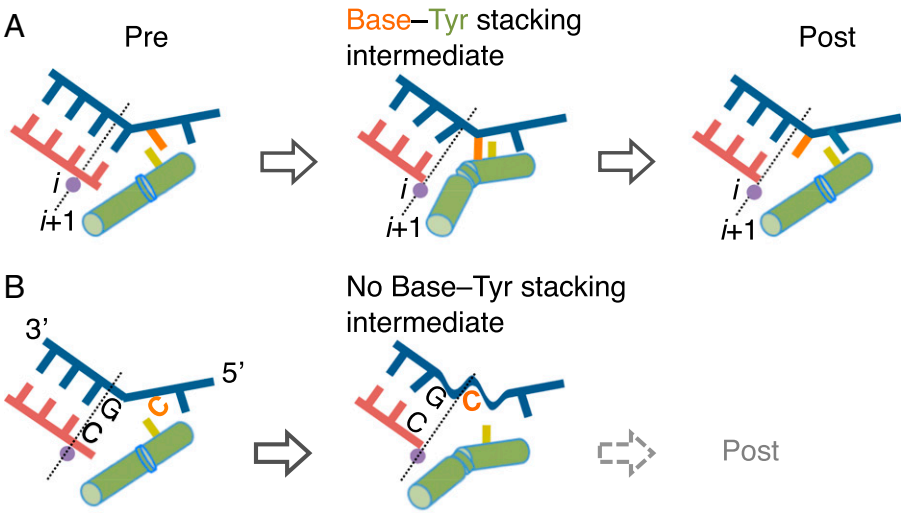

Fig. 1. A model for forward translocation proposed by Silva et al. (13). (*A*) Pause-free elongation and the DNA-base/Tyr836 stacking intermediate revealed by the MD simulation. RNA (red), template DNA strand (blue), catalytic Mg^{2+} (magenta circle), the transition template DNA base (orange), and the Tyr836 (yellow) in the BH (the bent and straight, green cylinder) in the pretranslocated and posttranslocated states are shown. The bent–straight transition of the BH is much faster than translocation (13). The base–Tyr stacking interaction is necessary for forward translocation in silico (13). (*B*) A model for pretranslocated pause. The 5′ CG 3′ sequence of the template DNA strand in the RNAP active center induces pausing (12). A highly flexible sugar backbone of dC residue at the CG junction (21) weakens the base–Tyr stacking, which hinders forward translocation.

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before one can use rate equations for modeling RNAP translocation (3, 18). For the same reason, applying the Markov process to construct the millisecond-long MD simulation of translocation needs additional validation (13). In the Markov process, probability distribution of the position of the reactant molecule at the time does not depend on the previously visited states. Therefore, the short-time memory effects on conformational changes during translocation become negligible. However, the memory effects may have a significant contribution when translocation occurs at low energy $\sim k_BT$ but requires a rare combination of sequential movements of the template DNA base, RNA–DNA hybrid, BH, and trigger loop. Translocation without significant activation energy and energy barrier $>>k_BT$ may be a common theme during pause-free transcription unless the opposite is proven. In this scenario, the stacking interaction between the DNA base and the Tyr836 may be beneficial for the geometry rather than energy barrier for the DNA base movement across the oscillating BH to determine the frequency of forward translocation. The energetic contribution of the RNA base–Tyr stacking interaction has been experimentally calculated as ∼2–5 k_BT at room temperature (19, 20), supporting this view.

Silva et al. (13) also provide an insight to the mechanism of transcription pausing due to a DNA-encoded translocation block (3). The robust pausing sites for *E. coli* RNAP and yeast/human RNAP II share a striking sequence similarity, as revealed by single-molecule and bulk biochemical studies (7, 10–12). Typically, the template DNA strand at the pause site contains a pyrimidine/purine sequence (e.g., 5′-CG-3′), where the dG is base-paired with the 3′ RNA end in the *i* site and the dC is base-paired with NTP in the *i*+1 site in the posttranlocated RNAP (Fig. 1*B*). Intriguingly, NMR studies have revealed that the CG step of double-strand DNA increases flexibility of the sugar-moiety of the cytosine residue (21). If this is also true for the RNA–DNA hybrid and the unpaired *i*+1 pyrimidine base at the pause site, the flexible sugar-backbone in the DNA strand surrounding the *i*+1 site may interfere with the stable base-Tyr836 stacking and prevent forward translocation (Fig. 1*B*). A similar phenomenon can be also responsible for misalignment of NTP in *i*+1 site during posttranslocation pausing of bacterial RNAPs (22), and for fraying of the 3′ RNA end in the hybrid from the template DNA strand in *i* or *i*+1 site during pretranslocation pausing in all kingdoms of life (3).

It is worth noting several inconsistencies between the results of MD simulations and those of the previous in vitro experiments. First, the former indicated that translocation and pyrophosphate release occur at ∼10 μs and ∼1 μs, respectively (13, 23). However, a biochemical study of *E. coli* RNAP revealed that both processes occur around 10 ms (24). This finding is consistent with single-molecule analyses of transcription indicating that (*i*) forward translocation of *E. coli* RNAP is the slowest process for the pause-free sequences tested (25), and (*ii*) translocation of yeast RNAP II also occurs in 10-ms rather than 10-μs order (26). This disagreement awaits solution by performing the MD simulation with structural models of RNAP in the pre-backtracked and backtracked states carrying a frayed and completely extruded 3′ RNA end, respectively. If forward translocation will not occur within 10-ms order in the MD simulation using these models, it would indicate that chemical energy is necessary for forward translocation. For example, the energy may be obtained from pyrophosphate release, which causes trigger loop opening, thereby preventing backward translocation (27).

Silva et al. identify an important component of the translocation mechanism using millisecond molecular dynamics simulation of translocation of yeast RNAP II.

Finally, all available X-ray structures of RNAPs use specially designed 8- or ≥ 9-bp RNA–DNA hybrids to stabilize the post- and the pretranslocated state, respectively (3). These scaffolds are incomplete and lack the nontemplate DNA forming the transcription bubble, the DNA duplex upstream of the hybrid, and single-stranded RNA upstream from the hybrid. As a result, some nucleic acid–protein interactions potentially involved in control of translocation will be missing in these complexes. It is known that the length and sequence of the RNA–DNA hybrid affect translocation (3). The hybrid may also fluctuate between 7- and 10-bp lengths, depending on transcribed sequence, the presence of secondary structures in the nascent RNA, and upon binding of transcription factors, all of which provide additional layers of translocation control (3). Thus, the translocation intermediates and the timescales identified by Silva et al. (13) will require further validation before application to the broad variety of sequence landscapes.

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