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Review

Translocation by multi-subunit RNA polymerases

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ABSTRACT

DNA template and RNA/DNA hybrid movement through RNA polymerase (RNAP) is referred to as "translocation". Because nucleic acid movement is coupled to NTP loading, pyrophosphate release, and conformational changes, the precise ordering of events during bond addition is consequential. Moreover, based on several lines of experimental evidence, translocation, pyrophosphate release or an associated conformational change may determine the transcription elongation rate. In this review we discuss various models of translocation, the data supporting the hypothesis that translocation rate determines transcription elongation rate and also data that may be inconsistent with this point of view. A model of the nucleotide addition cycle accommodating available experimental data is proposed. On the basis of this model, the molecular mechanisms regulating translocation and potential routes for NTP entry are discussed.

1. Structural features of the multi-subunit RNAPs

Multi-subunit RNA polymerases (RNAPs) are large, dynamic molecular machines ubiquitous to the three kingdoms of life, eubacteria, archaea and eukarya, that share a significant degree of homology [1–3]. A multitude of biochemical and structural evidence suggests that the basic mechanism of transcription elongation is common between RNAPs from different organisms. Therefore, most of the structural and functional properties determined for one multi-subunit RNAP are generally applicable to other multi-subunit RNAPs.

RNAPs extend a RNA chain by processively reading a DNA template strand [4]. In doing so, RNAPs function as powerful molecular motors [5] translocating along DNA and only releasing the template and its RNA transcript in response to specific termination signals [4,6,7]. In Fig. 1, images of the *Saccharomyces cerevisiae* RNAP II ternary elongation complex (TEC) [8] are shown. In contrast to DNA polymerases, which can release and re-bind DNA during replication, even temporary dissociation of the RNAP TEC leads to the collapse of the transcription bubble and abortion of transcription. To prevent this from happening, RNAPs encircle DNA with a protein clamp (Fig. 1A) limiting accessibility of the active center to incoming substrates and hindering release of inorganic pyrophosphate — a bi-product of phosphodiester bond formation. In this review, we discuss the mechanisms and structural elements in RNAP (protein channels, helices and flexible loops) that are involved in substrate delivery, catalysis, pyrophosphate release and translocation along DNA.

The long "bridge helix" (BH) [9,10], which borders the active site, is a defining feature of RNAP. Because the BH abuts the RNA/DNA hybrid, a possible molecular mechanism for translocation (Figs. 1B and 2) might involve thermal fluctuations and/or local unwinding or bending of the BH [9,11–14] (Fig. 3). Indeed, identification of hyperactive BH mutant RNAPs by saturation mutagenesis supports the potential importance of BH dynamics in translocation [14]. The two "trigger helices" (TH), connected by a short flexible "trigger loop" (TL), form a three-helix bundle with the BH. The TH–TL part of this bundle undergoes a major conformational change (TL→TH closing) upon binding of the NTP substrate to the active center of RNAP [15] (Fig. 1B). Notably, because of close proximity between TH and BH, opening and closing of the TL–TH is expected to modify BH dynamics and thus regulate translocation [11,13,14,16]. The existence of the specific conformation of the TL, wedged against the central part of the BH and stabilized by the transcription inhibitor α-amanitin [13], supports the possibility of a coordinated conformational change of the TL and BH during translocation (Fig. 3, structure 2). The concept of thermally driven translocation [11,13,17–22] has been a dominant theme in the field, with the basic idea that RNAP spontaneously steps between pre- and post-translocation states (Fig. 2).

Because the RNAP active site is buried deep within the RNAP structure, the question arises of the route(s) of NTP entry into the TEC. One possible route is the secondary pore, a solvent accessible channel [8,16,23–25] that appears to be gated by opening and closing of the TL [8], folding of the TL to the TH [16] and by the translocation state of the TEC [8,9] (Figs. 1 and 2). When loaded through the secondary pore, it appears that NTPs can bind only to the post-translocated TEC because,

Abbreviations: RNAP, RNA polymerase; TEC, ternary elongation complex; TFII, transcription factor RNA polymerase II; BH, bridge helix; TH, trigger helices; TL, trigger loop; FL2, fork loop 2; P, pre-insertion site; A, insertion site

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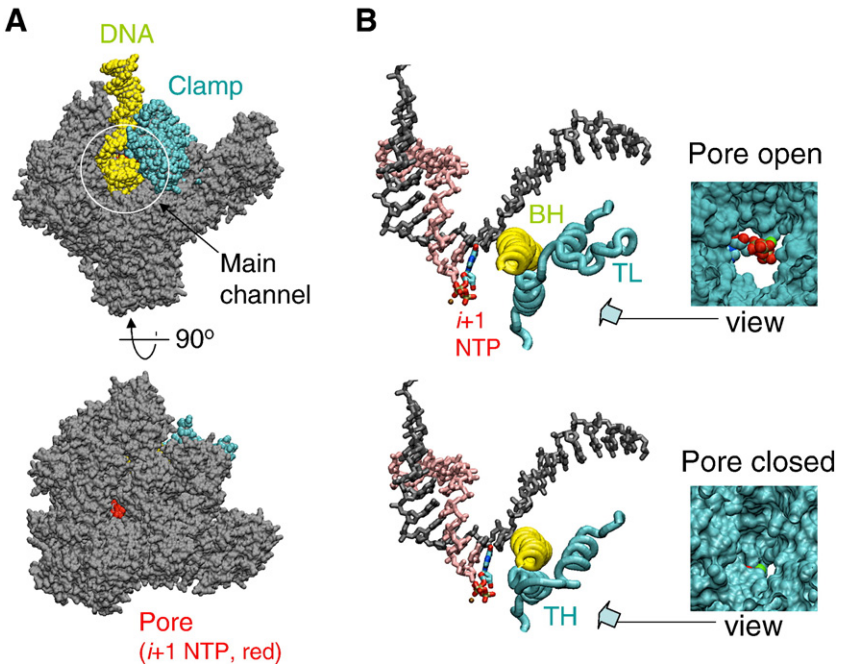

Fig. 1. Features of the RNAP TEC. A) *S. cerevisiae* RNAP II TEC. Protein, gray; "clamp", cyan; nucleic acids, yellow; NTP, red. B) Detailed images of the active site in open (above) and closed (below) TL–TH conformations. DNA template, gray; RNA, pink; NTP, chemistry (C, cyan; N, blue; O, red; P, tan); bridge helix, yellow; TL or TH, cyan. At the right are images of the secondary pore. Protein, cyan; NTP, chemistry; Mg^{2+}, green.

in the pre-translocated TEC, the DNA template base projects into the main enzyme channel rather than the secondary pore [9] (Fig. 2, upper panel). In addition, the close proximity of the 3′ end of the nascent transcript against the BH in the pre-translocated TEC (Figs. 2 and 3) leaves little space for the incoming NTP in the active center [9].

2. The role of translocation in the nucleotide addition cycle

Transcription elongation occurs by a repetitive nucleotide addition cycle. The TEC, ready for catalysis, has the 3′ end of the nascent RNA base paired to the template and positioned in the *i* site (Fig. 2, lower panel); the substrate NTP enters the active center and is paired to the next DNA base in the $i+1$ site. The NTP is positioned for catalysis by a conformational change (isomerization) of the RNAP. In single-subunit RNAPs, isomerization comprises a major closing of RNAP domains [26]; in multi-subunit RNAPs, isomerization includes movement of the TL, a mobile element of the catalytic subunit [8,16], TL folding into the TH [16], and formation of multiple contacts of the TH with the NTP in the RNAP active site (Fig. 3) [8,16]. The closed, catalytic TH structure has a primarily helical conformation (Fig. 3; structure 3), while the relaxed TL has substantially more loop (structures 1 and 2) [16]. Characterization of TL mutants [27,28] and kinetic analyses of

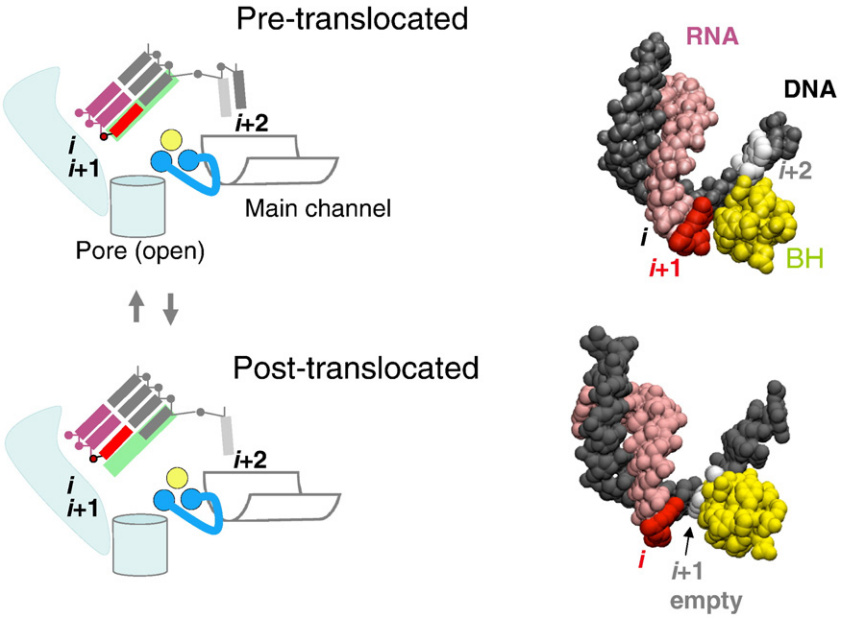

Fig. 2. Thermal ratchet translocation. DNA template, gray; RNA, pink and red; $i+1$ site, green shading; BH, yellow; TL in the open conformation, cyan. In the pre-translocated state, the 3′ end of the RNA (red) resides in the $i+1$ site. In the post-translocated state, the 3′ end of the RNA resides in the *i* site. The picture represents a view along the long axis of the BH. The right-side cartoon shows the space-filled representation of the structures of the *i* and $i+1$ sites in the pre-translocated and post-translocated TEC.