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RNA polymerase II transcriptional fidelity control and its functional interplay with DNA modifications

Liang Xu¹, Wei Wang¹, Jenny Chong¹, Ji Hyun Shin¹, Jun Xu¹, and Dong Wang^{1,*}

¹Skaggs School of Pharmacy and Pharmaceutical Sciences, The University of California San Diego, La Jolla, California, 92093-0625, United States

Abstract

Accurate genetic information transfer is essential for life. As a key enzyme involved in the first step of gene expression, RNA polymerase II (Pol II) must maintain high transcriptional fidelity while it reads along DNA template and synthesizes RNA transcript in a stepwise manner during transcription elongation. DNA lesions or modifications may lead to significant changes in transcriptional fidelity or transcription elongation dynamics. In this review, we will summarize recent progress towards understanding the molecular basis of RNA Pol II transcriptional fidelity control and impacts of DNA lesions and modifications on Pol II transcription elongation.

Keywords

Transcription fidelity; RNA polymerase II; DNA damage; Epigenetic DNA modifications; Structural biology; Chemical biology; Computational biology

1. Introduction

Transcription is the first key step for gene expression. RNA Polymerase II (Pol II) is the central enzyme responsible for pre-mRNA and noncoding RNA synthesis in eukaryotic cells (Kornberg, 2007). Transcriptional mutagenesis induced by damaged DNA templates may generate erroneous nascent mRNAs and as a result, lead to malfunction noncoding RNA and miscoded/misfolded proteins (Saxowsky and Doetsch, 2006). These transcription errors can have transient or heritable phenotypic changes for the cell, which may also be involved in tumour development (Bregeon and Doetsch, 2011; Gordon et al., 2009; Gordon et al., 2015; Morreall, Petrova, and Doetsch, 2013; Saxowsky and Doetsch, 2006; Viswanathan, You, and Doetsch, 1999). Another threat to the fidelity of RNA synthesis is from the nucleotide pool, in which the damaged ribonucleotides can be directly incorporated, inducing altered secondary or 3D structures of non-coding RNA transcripts or specificities of codon-anticodon recognition for incorporation of amino acids during translation (Bregeon and

*To whom correspondence should be addressed. Tel: 858 822 5561; Fax: 858 822 1953; ; Email: dongwang@ucsd.edu

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Doetsch, 2011). Therefore, maintaining an accurate genetic information transfer (high transcriptional fidelity) is essential for the process of life (Libby and Gallant, 1991).

RNA Pol II can be viewed as a highly specific “DNA reader” as well as an “RNA writer”. During transcription, Pol II moves along and recognizes the DNA template strand, and selects the matched nucleotide triphosphate (NTP) for synthesizing complementary RNA transcripts with a very low error rate (less than 0.001%) (Imashimizu et al., 2014; Kaplan, 2013; Liu, Bushnell, and Kornberg, 2013; Martinez-Rucobo and Cramer, 2013; Svejstrup, 2013; Svetlov and Nudler, 2013; Xu et al., 2014; Zhang and Wang, 2013). Pol II active site is highly evolved to select for the cognate substrate that matches the DNA template through a major conformational change of trigger loop to seal the active site and form an extensive interaction network with cognate substrate poised for nucleotide addition and to exclude non-cognate substrates (Kaplan, Larsson, and Kornberg, 2008; Kellinger et al., 2012; Kireeva et al., 2008; Wang et al., 2006; Yuzenkova et al., 2010). A combination of genetic, biochemical, and structural studies have provided extensive details on the molecular mechanisms of RNA polymerase transcription and its fidelity control in the last decade (Bregoon et al., 2003; Erie, Yager, and Vonhippel, 1992; Gnatt et al., 2001; Huang et al., 2010; Jeon and Agarwal, 1996; Kaplan, Larsson, and Kornberg, 2008; Kashkina et al., 2006; Kettenberger, Armache, and Cramer, 2003; Kettenberger, Armache, and Cramer, 2004; Kireeva et al., 2008; Koyama et al., 2007; Nesser, Peterson, and Hawley, 2006; Reines, Conaway, and Conaway, 1999; Thomas, Platas, and Hawley, 1998; Trinh et al., 2006; Walmacq et al., 2012; Wang et al., 2009; Wang et al., 2006; Westover, Bushnell, and Kornberg, 2004; Westover, Bushnell, and Kornberg, 2004; Yuzenkova et al., 2010). At least three fidelity checkpoints are utilized to ensure high transcriptional accuracy (Kellinger et al., 2012; Xu et al., 2014; Zhang and Wang, 2013). These fidelity checkpoints include insertion step (specific nucleotide selection and incorporation), extension step (from a matched vs a mismatched 3'-RNA terminus), and proofreading step (removal of misincorporated nucleotides from the 3'-RNA terminus) (Figure 1) (Kellinger et al., 2012; Xu et al., 2014; Yuzenkova et al., 2010; Zhang and Wang, 2013).

In addition to its critical roles in gene expression, RNA Pol II has also been proposed to be a highly selective sensor to detect abnormalities in DNA, such as DNA lesions, non-canonical DNA structures, and specific sequence motifs, and plays important roles in DNA damage tolerance and DNA repair (Belotserkovskii, Mirkin, and Hanawalt, 2013; Hanawalt and Spivak, 2008; Lindsey-Boltz and Sancar, 2007; Ljungman and Lane, 2004; Saxowsky and Doetsch, 2006; Tornaletti and Hanawalt, 1999). In fact, all cells and organisms constantly face dreadful threats of genomic DNA lesions caused by endogenous factors and environmental agents (Hoeijmakers, 2009; Jackson and Bartek, 2009). Every day, there are more than one million DNA lesions generated in a single cell (Lindahl and Barnes, 2000), and some of them cause significant DNA structural and chemical alterations. These alterations within highly transcribed genomic regions would significantly interfere with Pol II transcription process (Hanawalt and Spivak, 2008; Laine and Egly, 2006; Saxowsky and Doetsch, 2006; Svejstrup, 2007). Biochemical and genetic studies have shown that DNA damage processing by Pol II is lesion-specific (Hanawalt and Spivak, 2008; Lagerwerf et al., 2011; Lindsey-Boltz and Sancar, 2007; Saxowsky and Doetsch, 2006; Svejstrup, 2007; Tornaletti and Hanawalt, 1999). Pol II can bypass, backtrack, or stall at DNA lesions,

leading to distinct downstream consequences. Pol II transcriptional lesion bypass is sometimes associated with misincorporation within the RNA transcript, termed transcriptional mutagenesis (Doetsch, 2002; Saxowsky and Doetsch, 2006). On the other hand, Pol II stalling at bulky DNA lesions initiates a specialized DNA repair pathway, termed transcription-coupled repair (TCR) (Hanawalt and Spivak, 2008; Lagerwerf et al., 2011; Saxowsky and Doetsch, 2006; Tornaletti and Hanawalt, 1999), which preferentially removes DNA lesions in the transcribed strand (Bohr et al., 1985; Foustari and Mullenders, 2008; Hanawalt and Spivak, 2008; Lagerwerf et al., 2011; Mellon et al., 1986; Mellon, Spivak, and Hanawalt, 1987; Sarker et al., 2005). Arrested Pol II by DNA lesion is proposed as the initial signal for the recruitment of repair proteins involved in TCR though the detailed recruiting mechanism remains to be elucidated (Malik et al., 2010; Sarker et al., 2005; Troelstra et al., 1993; Troelstra et al., 1992; van den Boom et al., 2004; van Gool et al., 1997). Defects in genes involved in TCR cause premature aging and human diseases, such as Cockayne Syndrome, Xeroderma Pigmentosum, Trichothiodystrophy, UV-sensitive syndrome, and Cerebro-Oculo-Facio-Skeletal Syndrome (Hanawalt, 2008; Hanawalt and Spivak, 2008; Jaakkola et al., 2010; Laugel et al., 2010). Finally, persistently arrested Pol II undergoes ubiquitylation and subsequent degradation (Lindsey-Boltz and Sancar, 2007; Svejstrup, 2007; Wilson, Harreman, and Svejstrup, 2013).

In this review, we will focus on discussing recent progresses that provide novel insights into the molecular basis and chemical perspectives of Pol II transcriptional fidelity control during transcription elongation through structural, computational and chemical biology approaches. In addition, we will discuss the current views of the functional interplay between Pol II transcriptional machinery and DNA modifications and lesions.

2. A combined approach to understand the molecular basis of Pol II transcriptional fidelity

A combination of genetic, biochemical, and structural studies have shed new light on the Pol II transcription mechanism at an atomic level over the last decade (Bregeon et al., 2003; Erie, Yager, and Vonhippel, 1992; Gnat et al., 2001; Huang et al., 2010; Jeon and Agarwal, 1996; Kaplan, Larsson, and Kornberg, 2008; Kashkina et al., 2006; Kettenberger, Armache, and Cramer, 2003; Kettenberger, Armache, and Cramer, 2004; Kireeva et al., 2008; Koyama et al., 2007; Nesser, Peterson, and Hawley, 2006; Reines, Conaway, and Conaway, 1999; Thomas, Platas, and Hawley, 1998; Trinh et al., 2006; Walmacq et al., 2012; Wang et al., 2009; Westover, Bushnell, and Kornberg, 2004; Westover, Bushnell, and Kornberg, 2004; Yuzenkova et al., 2010; Yuzenkova and Zenkin, 2010; Zhang et al., 2006). Several excellent reviews focusing on Pol II enzymatic catalysis and transcriptional regulation were published elsewhere (Kaplan, 2013; Liu, Bushnell, and Kornberg, 2013; Martinez-Rucobo and Cramer, 2013; Svejstrup, 2013; Svetlov and Nudler, 2013; Zhang and Wang, 2013). In this section, we will focus on recent progress towards understanding of mechanisms of three key fidelity checkpoint steps in controlling Pol II transcriptional fidelity at the elongation phase.

2.1 Structural biology studies

The first crystal structure of Pol II transcribing elongation complex was captured in a ‘pre-translocation’ state in which the Pol II active site is still occupied by the newly incorporated nucleotide (Gnatt et al., 2001). This crystal structure revealed several important structural features: RNA and DNA are held by the Pol II clamp in a closed conformation; the template DNA bends and crosses over the bridge helix to be delivered into the Pol II active site; the RNA forms a short upstream RNA-DNA hybrid as an intermediate between A and B form with the template DNA strand extending from the Pol II active site. The “pre-translocation” state is the state immediately after nucleotide addition. In order to make the active site available for the next round of nucleotide addition, Pol II must translocate one base pair downstream along the DNA template into a new state, termed the “post-translocation” state (Figure 2a). The Pol II complex in a post-translocation state was solved using a synthetic scaffold (Westover, Bushnell, and Kornberg, 2004; Westover, Bushnell, and Kornberg, 2004). Intriguingly, Pol II protein in the post-translocation state has an almost identical conformation with that in the pre-translocation state.

The availability of Pol II elongation complex structure in a post-translocation state also promotes us to address the essential question related to transcriptional fidelity control: how is the nucleotide substrate selected and bound to the Pol II active site? A series of crystal structures of Pol II elongation complexes with matched or mismatched substrates bound in the active site were determined (Kettenberger, Armache, and Cramer, 2004; Westover, Bushnell, and Kornberg, 2004; Westover, Bushnell, and Kornberg, 2004; Zhang et al., 2006). These structural studies provide the molecular basis of nucleotide selection and addition by Pol II, the first checkpoint step of transcriptional fidelity (Figure 2b). Intriguingly, only the matched NTP binds to the canonical “addition site” (or A site), in which the nucleobase of substrate forms a Watson-Crick base pair with the DNA template base (Figure 3a), whereas the mismatched NTP binds to an inverted conformation away from the DNA template, referred to as the “entry site” or E site (Hanawalt and Spivak, 2008; Kettenberger, Armache, and Cramer, 2003; Wang et al., 2006; Westover, Bushnell, and Kornberg, 2004). Importantly, upon the binding of the matched NTP, a conserved motif of Pol II, termed the trigger loop, switches (and folds) from an inactive open state to an active closed state (Figure 3a and 3b)–(Wang et al., 2006). Like many other nucleic acid enzymes, the closure of the active site is a common strategy to facilitate its enzymatic efficiency and selectivity (Beard and Wilson, 2006; Cramer, 2002; Doublet, Sawaya, and Ellenberger, 1999). Indeed, mutations in the trigger loop and nearby active site, or blockage of trigger loop movement by alpha-amanitin significantly affect Pol II transcription fidelity and catalytic efficiency (Braberg et al., 2013; Kaplan, Larsson, and Kornberg, 2008; Kireeva et al., 2008; Larson et al., 2012; Nediaklov et al., 2013; Strathern et al., 2012; Tan et al., 2008; Touloukhonov et al., 2007; Walmacq et al., 2012; Walmacq et al., 2009; Wang et al., 2006). Pol II also shares a universal two-metal ion catalytic mechanism with other nucleic acid enzymes (Figure 3a and 3b) (De Vivo, Dal Peraro, and Klein, 2008; Feng, Dong, and Cao, 2006; Schmidt et al., 2010; Sosunov et al., 2003; Steitz and Steitz, 1993). All three moieties of the substrate (nucleobase, sugar, and triphosphate) are recognized by Pol II through an extensive substrate recognition network (Wang et al., 2006). The nucleobase of the substrate is base paired with the DNA template and sandwiched by the 3'-RNA primer and Pol II trigger loop. These interactions ensure the

correct nucleobase positioning in both lateral and vertical directions (Huang et al., 2010; Wang et al., 2006). In sharp contrast, the mismatched nucleotide is bound to the “E site”, in which the trigger loop is in the inactive open conformation. Therefore, the rate of mismatched nucleotide incorporation (trigger loop independent nucleotide addition) is several orders of magnitude slower than the rate of correct substrate incorporation (trigger loop dependent nucleotide addition) (Kellinger et al., 2012; Yuzenkova et al., 2010). This key mechanistic difference contributes to the high transcriptional fidelity of insertion step.

Structural studies also provide insights into the molecular basis of the second fidelity checkpoint step. In this step, RNA transcript extension from a mismatched 3'-RNA terminus is extremely slower than the extension from a fully matched end. Indeed, the fully matched upstream RNA:DNA hybrid and nearby active site residues are essential for the proper alignment of the 3'-RNA terminus with active site metal ions and incoming NTP. In the Pol II structures containing a mismatched RNA terminus, the mismatched 3'-RNA terminus either adopts frayed states or causes Pol II to move in a reverse direction into backtracked states, extruded toward the “secondary channel” of Pol II, which is the same channel for NTP diffusion (Figure 3c) (Cheung and Cramer, 2011; Sydow et al., 2009; Wang et al., 2009). Consequently, the mismatched 3'-RNA terminus is in the inactive conformation and partially overlaps with the canonical substrate binding site. The next round of nucleotide binding and addition is greatly compromised.

Pol II proofreading activity is important for the removal of the mismatched nucleotide in the 3'-RNA terminus. This proofreading mechanism enables Pol II to cleave the dinucleotide containing a mismatched 3'-RNA terminus through backtracking-dependent proofreading mechanism to regenerate a new post-translocation state, allowing Pol II to have a “second chance” to reselect the correct nucleotide for incorporation and elongation. In fact, slow forward extension from the mismatched 3'-RNA terminus also provides a time window that allows for Pol II backtracking and proofreading (Cheung and Cramer, 2011; Jeon and Agarwal, 1996; Kalogeraki et al., 2005; Koyama et al., 2007; Thomas, Platas, and Hawley, 1998; Wang et al., 2009). Structural studies of backtracked Pol II provide a detailed understanding of these backtracked states and proofreading mechanisms. The single stranded 3'-RNA terminus containing the misincorporated nucleotide extrudes toward the secondary channel (Figure 3c), or the same channel for NTP diffusion and binding. Dinucleotides or short oligomers containing the misincorporated nucleotide are preferentially removed (Cheung and Cramer, 2011; Kettenberger, Armache, and Cramer, 2003; Walmacq et al., 2012; Wang et al., 2009). Two independent proofreading mechanisms are found: intrinsic cleavage and TFIIS-mediated cleavage (Figure 3c and 3d) (Awrey et al., 1997; Cheung and Cramer, 2011; Erie et al., 1993; Izban and Luse, 1993; Jeon and Agarwal, 1996; Johnson and Chamberlin, 1994; Kalogeraki et al., 2005; Kettenberger, Armache, and Cramer, 2004; Koyama et al., 2003; Koyama et al., 2007; Reines, Chamberlin, and Kane, 1989; Rudd, Izban, and Luse, 1994; Sigurdsson, Dirac-Svejstrup, and Svejstrup, 2010; Thomas, Platas, and Hawley, 1998; von Hippel, 1998; Wang et al., 2009; Weilbaeher et al., 2003; Yuzenkova et al., 2010; Zenkin, Yuzenkova, and Severinov, 2006). In the intrinsic cleavage mode (Figure 3c), Pol II removes one or two nucleotides from the 3'-RNA terminus without the recruitment of external transcription factors. A metal bound water molecule from free solution (Figure 3c) is expected to attack the phosphodiester backbone of RNA

transcript for intrinsic cleavage. On the other hand, for TFIIIS-stimulated cleavage mode, transcription factor TFIIIS is recruited to the backtracked complex to stimulate the transcript cleavage in the case of extensive backtracking (Cheung and Cramer, 2011; Kettenberger, Armache, and Cramer, 2003; Kettenberger, Armache, and Cramer, 2004; Powell, Bartholomew, and Reines, 1996; Wang et al., 2009) (Figure 3d). The domain III of TFIIIS is inserted into the Pol II active site and provides residues for chelating magnesium ions that is important for cleavage (Figure 3d). The inserted domain III of TFIIIS also pushes Pol II trigger loop into an open (inactive) conformation. Therefore, to re-enter the productive Pol II elongation mode, the inserted domain III of TFIIIS needs to be displaced from the secondary channel, allowing the Pol II trigger loop to access its closed conformation during nucleotide addition cycles (Cheung and Cramer, 2011; Kettenberger, Armache, and Cramer, 2003; Kettenberger, Armache, and Cramer, 2004; Powell, Bartholomew, and Reines, 1996; Wang et al., 2009; Wang et al., 2006). Multiple rounds of backtracking, cleavage, and reactivation could happen when Pol II encounters DNA lesions or translocation barriers (Awrey et al., 1998; Awrey et al., 1997; Erie et al., 1993; Izban and Luse, 1993; Jeon and Agarwal, 1996; Johnson and Chamberlin, 1994; Kalogeraki et al., 2005; Kettenberger, Armache, and Cramer, 2004; Koyama et al., 2003; Koyama et al., 2007; Reines, Chamberlin, and Kane, 1989; Rudd, Izban, and Luse, 1994; Sigurdsson, Dirac-Svejstrup, and Svejstrup, 2010; Thomas, Platas, and Hawley, 1998; von Hippel, 1998; Wang et al., 2009; Weilbaecher et al., 2003; Yuzenkova et al., 2010; Zenkin, Yuzenkova, and Severinov, 2006). This provides a mechanism for increasing overall transcriptional bypass by either suppressing DNA-lesion induced transcriptional mutagenesis or providing enough time for the recruitment of DNA repair machinery.

2.2 Revealing Pol II transcriptional dynamics through computational biology approaches

While the crystal structures of Pol II elongation complexes have provided atomic structural snapshots of Pol II transcription machinery at different states (Cheung, Sainsbury, and Cramer, 2011; Cramer, Bushnell, and Kornberg, 2001; Gnatt et al., 2001; Kornberg, 2007; Wang et al., 2009; Wang et al., 2006), little is known about the transcriptional dynamics or the detailed conformational transition from one state to another (Brueckner et al., 2009; Svetlov and Nudler, 2009). Molecular dynamics (MD) simulations provide us a powerful complementary approach to gain novel understandings of the dynamic transcription process (Batada et al., 2004; Da, Wang, and Huang, 2012; Feig and Burton, 2010; Feig and Burton, 2010; Huang et al., 2010; Kireeva et al., 2012). Indeed, the great advances in computational power, algorithms, and force field in the last decade allow us to apply molecular dynamics simulation to study the Pol II elongation complex in a biologically relevant time scale. Here we review some of the recent computational studies, mainly focusing on NTP loading and recognition, as well as pyrophosphate ion (PP_i) release and translocation.

Crystal structures revealed multiple nucleotide binding sites (entry site, addition site, and pre-insertion site as intermediates). However, it is not clear how and through which routes nucleotides diffuse from free solution to the Pol II active site or how pyrophosphate releases from active site to solution. Batada *et al* addressed the NTP diffusion question using a computational biology approach (Batada et al., 2004) and revealed a functional role for the “entry site” in facilitating NTP diffusion into the “addition site” (Figure 4). Once the NTP

reaches the addition site, it is further recognized and stabilized by the closed trigger loop via several specific interactions between the NTP and trigger loop residues (Wang et al., 2006). The functional roles of trigger loop residues (such as Leu1081 and His1085 (Rpb1)) in positioning and stabilizing the NTP were systematically evaluated by all-atom MD simulations (Huang et al., 2010). The MD simulations revealed that the protonated His1085 is the most favorable form when interacting with the β -phosphate atom of NTP. Both H1085F and H1085Y mutants can compromise the interaction between NTP and the trigger loop. These computational results are consistent with the experimental observations that H1085Y can induce cell growth defects and H1085F (or H1085A) is lethal to the cell (Braberg et al., 2013; Irvin et al., 2014; Larson et al., 2012; Touloukhonov et al., 2007). In addition, the authors suggested that hydrophobic interactions between Leu1081 of the side chain and the nucleotide base of the incoming NTP is important for stabilizing the nucleotide base in the correct position.

The PP_i release after nucleotide incorporation is the least understood step in nucleotide addition cycle. Several important questions remain to be addressed. How does the PP_i release along the secondary channel? What is the relationship between the PP_i release and the opening motion of the trigger loop (TL)? The kinetics of PP_i release and its possible release paths were recently investigated using MD simulations (Da, Wang, and Huang, 2012). Intriguingly, the MD simulations results suggest that PP_i adopts a hopping model through several conserved positively charged residues of Pol II to release from the active site to the secondary channel. Within each hopping site, (Mg-PP_i)²⁻ can form favorable interactions with Pol II and greatly increase its residence time. Single mutant simulations further suggest that H1085 and K752 (Rbp1) aid PP_i exit from the active site after catalysis, whereas K619 facilitates its passage through the secondary channel. In addition, the authors reported that PP_i release was tightly coupled with the trigger loop tip motion, but the dynamics of PP_i release is much faster than the full opening of trigger loop and translocation (Figure 4). Therefore, these results argued that PP_i release cannot be directly coupled with translocation (so-called power stroke model), which in general occurs in longer timescales than PP_i release (Malinen et al., 2012). This study provides novel mechanistic insights into the dynamics of PP_i release.

How RNA Pol II translocates along the DNA template is a long-standing question in the transcription field. Very limited mechanistic insights can be obtained from structural studies, as the structures of Pol II elongation complexes in pre- and post- translocation states are virtually identical except the RNA at the 3'-terminus. Even for computational biology approach, direct simulation of Pol II translocation at the biologically relevant timescale (milliseconds) for such a huge system (nearly half a million atoms) is not yet possible even with specialized simulation hardware. Recently, Huang, Wang, and colleagues used Markov State Models to overcome the "timescale gap" of conventional simulations (Silva et al., 2014). Intriguingly, their simulations revealed that RNA Pol II translocation is driven by thermal energy and does not require input of any additional chemical energy. Furthermore, the authors identified two novel metastable translocation intermediate states that are too transient to be captured by structural studies. The authors further suggested an important role for the bridge helix in facilitating translocation by lowering the free energy barriers. This dynamic view of translocation, though built on the mini-scaffold lacking a full

transcriptional bubble and in the absence of NTP, represents a substantial advance over the current understanding based on static snapshots provided by X-ray structures. It would be of great interest to simulate Pol II transcription with a full transcriptional bubble and in the presence of NTP to fully recapitulate the Pol II translocation process in the near future.

2.3 Understanding chemical interactions and intrinsic structural features in controlling Pol II transcriptional fidelity by synthetic nucleic acid analogues

Previous structural studies revealed a substrate recognition network with multiple types of chemical interactions including base stacking, hydrogen bonding, hydrophobic interactions, and salt bridge interactions (Huang et al., 2010; Wang et al., 2006). However, it is unclear how each of these interactions contributes to the transcriptional fidelity of Pol II. Dissecting individual contributions of these chemical interactions will provide insight into the molecular mechanism by which Pol II reads the DNA template and how DNA modifications and lesions will affect transcriptional fidelity by altering the chemical functional groups and structural features of nucleic acids.

We recently employed a chemical biology approach to systematically examine the individual contributions of chemical interactions (such as hydrogen bonds and base stacking) and nucleic acid structural motifs in Pol II transcriptional fidelity control. In collaboration with the Kool group, a series of synthetic ‘hydrogen-bond deficient’ nucleoside analogues were used as chemical probes to dissect hydrogen-bonding interactions governing RNA Pol II transcriptional fidelity (Kellinger et al., 2012). Nonpolar thymidine analogues were synthesized to mimic the shape of thymine nucleoside with overall 1.0 Å increment in size from the smallest dH to the largest dI (Figure 5a) (Kim et al., 2005; Kim and Kool, 2005). This system allows us to measure the effects of the loss of hydrogen bonding at the individual “checkpoint” steps (Khakshoor et al., 2012). In addition, the size effect of the template nucleobase can be evaluated by comparing among the five analogues.

This study reveals distinct contributions of hydrogen bonds at each step of the fidelity checkpoints. In the insertion step, Pol II loses the discrimination power between ATP and UTP for the dF, dL, dB, and dI templates, indicating the significant role of hydrogen bonding in ensuring correct substrate incorporation in the first fidelity checkpoint step. This is in sharp contrast to some of the high fidelity DNA polymerases where dNTP incorporation is relatively less affected by hydrogen bond deficiency. Unexpectedly, we revealed a different role of hydrogen bonding during the extension step. The loss of hydrogen bonds between the matched 3'-terminus base pair does not drastically affect CTP incorporation specificity. More strikingly, we observed significant increases (~100–1000 fold) of extension from a mismatched pair upon the removal of the wobble hydrogen bonding. These results suggest the unexpected contribution of wobble pair hydrogen bonding in preventing mismatched extension. These two different aspects indicate that while the canonical hydrogen bonding assures the correct nucleotide insertion in the first fidelity checkpoint step, the wobble hydrogen bonding is important in preventing mismatch extension in the second fidelity checkpoint step. In the third checkpoint step of Pol II transcription, we found that loss of Watson-Crick hydrogen bonds leads to a significant increase in backtracking and cleavage activities. Interestingly, nonpolar analogues with

larger sizes (dB and dI) can compensate for the loss of hydrogen bonds at the 3'-end of RNA, presumably by increased base stacking that could strengthen the RNA:DNA duplex stability. Taken together, this study systematically evaluated the effects of hydrogen bonding as well as contributions of nucleobase size and stacking at individual transcriptional fidelity checkpoint steps.

Wang, Wengel, and co-workers further investigated the contribution of the sugar backbone to transcriptional fidelity using sugar analogues (Xu et al., 2013). The sugar backbone is the central structural moiety connecting all of the peripheral nucleic acid functional groups: the nucleobase, 2'- and 3'-OH, and phosphate groups. However, the contribution of this core moiety in maintaining accurate genetic information transfer is unclear. To reveal the contribution of the sugar backbone to Pol II transcriptional fidelity, a "sugar backbone mutant" ribonucleotide analogue, termed unlocked nucleoside (UNA), was selected for comparative studies (Figure 5b) (Langkjaer, Pasternak, and Wengel, 2009). The UNA analogue contains all of the same peripheral functional groups of the "wild type" ribonucleotide except that it misses a bond that connects the C2' and C3' atoms of the ribose ring. Importantly, UNA residues maintain the capability to form Watson-Crick base pairing within DNA strands and form a similar duplex structure in comparison with a RNA/DNA hybrid (Pasternak and Wengel, 2010). We found that the sugar backbone is an underappreciated, dominant factor in controlling all three checkpoint steps of Pol II transcriptional fidelity (Xu et al., 2013). Nucleobase discrimination is completely abolished following the replacement with the unlocked sugar in every single fidelity checkpoint step. In fact, the sugar backbone integrity is a prerequisite for correct nucleotide selection in Pol II transcription. Pol II has an unexpectedly strong discrimination power for interrogating substrate ribose integrity, which is even ~100-fold stronger than its ability to discriminate against a mismatched base and 10^3 – 10^4 stronger than its ability to discriminate against dNTPs (Xu et al., 2013). Therefore, the correct peripheral functional groups of the nucleotide *per se* are insufficient for efficient Pol II transcription. Rather, the correct spatial arrangement of peripheral functional groups (restrained by the sugar backbone) is essential in maintaining high Pol II catalytic activity and fidelity. Indeed, the Pol II active site is not fully pre-assembled and the binding of the nucleotide substrate with the correct spatial arrangement of its functional groups is important for closure of the trigger loop and full assembly of Pol II active site poised for catalysis (Kaplan, Larsson, and Kornberg, 2008; Malinen et al., 2012; Yuzenkova et al., 2010; Zhang et al., 2006).

Phosphodiester linkage is also critical for maintaining RNA Pol II transcriptional fidelity. The non-enzymatic RNA polymerization introduces backbone heterogeneity with a mixture of 2'-5' and 3'-5' linkages (Figure 5c) (Bowler et al., 2013; Ekland and Bartel, 1996; Ertem and Ferris, 1996; Ferris and Ertem, 1992; Inoue and Orgel, 1982; Usher and McHale, 1976; Usher and McHale, 1976). Pol II is a key modern enzyme responsible for synthesizing 3'-5'-linked RNA with high fidelity. It is not clear how Pol II selectively recognizes the 3'-5' linkage over 2'-5' linkage. Recently, Wang and collaborators systematically investigated how phosphodiester linkages of nucleic acids govern Pol II transcriptional efficiency and fidelity (Xu et al., 2014). Intriguingly, it was revealed that Pol II recognizes phosphodiester linkages in an asymmetric (strand-specific) manner. These results reveal essential contributions of the template phosphodiester linkages to Pol II transcription and provide new

understanding on nucleic acid recognition and genetic information transfer during molecular evolution. Intriguingly, Pol II stalls at a 2'-5' linkage in a strikingly similar manner as a CPD lesion or Cyclo-dA lesion, suggesting a likely common recognition mode for these three modifications (Walmacq et al., 2012; Walmacq et al., 2015).

The nucleic acid analogues are useful to probe specific interactions between Pol II functional domains and nucleic acid moieties. Wang and collaborators recently utilized a series of synthetic nucleotide analogues and alpha-amanitin to reveal the chemical interactions and substrate structural signatures governing Pol II trigger loop closure (Xu et al., 2014). Investigations of hydrogen bonding deficient nucleotide analogues suggested that the formation of Watson-Crick hydrogen bonds is dispensable in promoting trigger loop closure, and the loss of hydrogen bonds can be fully rescued by increasing the size (base stacking) of a nucleobase. In addition, we also found the sugar pucker ring conformation plays a much more important role in facilitating nucleotide incorporation and trigger loop closure than that of hydrogen bonds via 2'-OH. Employing synthetic nucleotide analogues, this study highlighted the important contributions of π - π stacking (base stacking) and van der Waals interactions (sugar pucker) in governing the Pol II substrate recognition and full trigger loop closure.

Collectively, these studies using synthetic nucleic acid analogues highlight the importance of chemical interactions and the intrinsic structural features (backbones) of nucleic acids in controlling Pol II transcriptional fidelity that could not be achieved by conventional biological observations and analyses (Benner, 2004; Benner and Sismour, 2005; Kellinger et al., 2012; Khakshoor and Kool, 2011; Xu et al., 2014; Xu et al., 2013; Zhang and Wang, 2013). These studies also provide a framework for understanding the effect of disrupted chemical interactions and structural features by DNA lesions on Pol II transcriptional fidelity. This synthetic chemical biology approach may be extended to understand the mechanisms of other RNA polymerases as well as other nucleic acid enzymes in future studies.

3. DNA lesion-induced transcriptional mutagenesis

Pol II can bypass certain types of DNA lesions in either an error-free or error-prone manner (Doetsch, 2002; Saxowsky and Doetsch, 2006; Scicchitano, 2005; Scicchitano, Olesnický, and Dimitri, 2004; Tornaletti, 2005; Wang et al., 2010; Waters et al., 2009; Xu et al., 2015). Error-prone transcription bypass may lead to miscoded mRNA or malfunctioning non-coding RNAs (Bregoon and Doetsch, 2011; Doetsch, 2002; Liu, Zhou, and Doetsch, 1995). Impacts of several common DNA lesions on Pol II transcription are thoroughly summarized and reviewed elsewhere (Hanawalt and Spivak, 2008; Saxowsky and Doetsch, 2006; Scicchitano, 2005; Scicchitano, Olesnický, and Dimitri, 2004; Tornaletti, 2005). The effects of DNA lesions on transcriptional fidelity appear to be lesion-specific and position-specific. Using alkylation damage as an example, different alkylated nucleotides or different alkylation positions in the same nucleotide may cause distinct consequences during transcriptional lesion bypass (Cline et al., 2004; Dimitri et al., 2008; Dimitri et al., 2008; You et al., 2015; You et al., 2014).

In recent years, there has been substantial progress towards understanding how pol II recognizes and processes DNA lesions, which also provides structural mechanistic insights into the impact of DNA lesions on transcriptional fidelity (error-prone vs error-free modes). Here we summarize some recent progresses of structural studies in this area, focusing on the following categories of DNA lesions and modifications: oxidative DNA lesions, 1,2-intrastrand DNA lesions, monofunctional bulky DNA lesions, and epigenetic DNA modifications.

3.1 Oxidative DNA lesion-induced transcriptional mutagenesis

Oxidative DNA damage is one of the most abundant genomic DNA lesions induced by reactive oxygen species (ROS). Among these oxidative lesions, 8-oxo dG is well documented as a mutagenic DNA lesion (Saxowsky et al., 2008). Pol II can bypass this lesion and incorporate either a matched cytosine or a mismatched adenine opposite 8-oxo dG (Kuraoka et al., 2007; Tornaletti et al., 2004). Structural studies of Pol II with a template containing a site-specific 8-oxo dG confirmed that 8-oxo dG adopts a canonical *anti* conformation when paired with a cytosine as a Watson Crick base pair, and a *syn* conformation to form a Hoogsteen base pair with adenine (Figure 6a) (Damsma and Cramer, 2009). Therefore, the presence of an 8-oxo dG-lesion leads to great increases of efficiencies of ATP mis-insertion and extension step from an 8-oxo dG:rA base pair. Meanwhile, the presence of 8-carbonyl group reduces the stability of an 8-oxo dG at *anti* conformation and thus reduces the efficiencies of cognate CTP insertion and extension. Taken together, the combined effects cause an error-prone transcriptional bypass. Notably, Pol II essentially shares the same mechanism of adenine misincorporation at 8-oxo dG with some high fidelity DNA polymerases (Briebe et al., 2004; Eoff et al., 2007). These results suggest that, at least in the case of 8-oxo dG, the DNA lesion-induced changes in the structural features of nucleic acids play a very important role in controlling the potential functional outputs for RNA polymerases and high fidelity DNA polymerases.

3.2. 1,2-intrastrand DNA lesions cause strong Pol II blockage and transcriptional mutagenesis

UV-induced Cyclobutane pyrimidine dimer (CPD) lesion and cisplatin-induced 1,2-dGpG intrastrand cross-links are two well-studied 1,2-intrastrand DNA lesions that greatly distort the DNA duplex structure (Figure 7) (Park et al., 2002; Todd and Lippard, 2009). These two DNA lesions strongly block Pol II transcription and initiate transcription-coupled repair (Corda et al., 1993; Cullinane et al., 1999; Donahue et al., 1994; Jung and Lippard, 2006; Kalogeraki et al., 2005; Kalogeraki, Tornaletti, and Hanawalt, 2003; Lee et al., 2002; Mei Kwei et al., 2004; Mello, Lippard, and Essigmann, 1995; Mellon, Spivak, and Hanawalt, 1987; Tornaletti et al., 1997; Tornaletti et al., 2003; Tornaletti, Reines, and Hanawalt, 1999; Wang and Lippard, 2005). Interestingly, structural studies of Pol II complexes stalled at the intrastrand cross-links caused by CPD lesions or cisplatin-induced DNA lesions suggested somewhat different mechanisms of Pol II recognition and processing of these two 1,2-intrastrand DNA lesions (Figure 7a and 7b) (Brueckner et al., 2007; Damsma et al., 2007; Walmacq et al., 2012; Wang et al., 2006). CPD lesions are less bulky than cisplatin-induced DNA lesions. Therefore, CPD lesions can be delivered into the Pol II active site. Indeed, a structure captures a CPD-stalled intermediate state in which the CPD lesion is

accommodated right above the bridge helix and partially reaches into the +1 site (PDB ID 4A93). The presence of a CPD DNA lesion greatly distorts the DNA template and therefore, a CPD lesion is a poor template to support template-dependent AMP incorporation opposite to 3'-thymine. Further translocation of the CPD lesion from +1 to -1 position is strongly disfavored. UMP misincorporation opposite the 5'-thymine is also reported (Figure 7a) (Brueckner et al., 2007; Walmacq et al., 2012). In contrast, the structure of the Pol II complex stalled at a 1,2-dGpG cisplatin-induced DNA lesion revealed that the lesion is accommodated at the +2/+3 position, and no stable structure of Pol II complex with a cisplatin-induced DNA lesion at position +1/+2 is captured. Indeed, the designed scaffold with a cisplatin-induced DNA lesion at position +1/+2 strongly favors backtracking and moves the cisplatin-induced DNA lesion at +2/+3 position. These results indicate the cisplatin-induced DNA lesion might be too bulky to cross over the bridge helix and be delivered into the active site (Figure 7b). Slow non-template dependent AMP misincorporation is observed opposite the cisplatin-induced DNA lesion.

3.3. Pol II blockage and bypass at monofunctional bulky DNA lesions

Unlike bifunctional cisplatin that forms 1,2-dGpG DNA lesions, pyriplatin (cis-diamminepyridinechloroplatinum(II)) and phenanthriplatin (cis-diamminephenanthridinechloroplatinum(II)) (Figure 8a) form monofunctional bulky platinum-induced DNA lesions at dG and dA sites on the DNA template without distorting the DNA duplex structure. These monofunctional platinum compounds may escape detection from DNA damage recognition proteins XPC/HR23B and subsequent global nucleotide excision repair. These compounds have also demonstrated unique anticancer activities against cisplatin-resistant cancer cell lines (Park et al., 2012). It is therefore particularly interesting to understand how these monofunctional platinum compounds accommodate within the Pol II active site and affect Pol II transcription.

The structural studies of Pol II complex stalled at a pyriplatin-DNA monofunctional adduct revealed novel insights into how Pol II recognizes and processes this bulky monofunctional platinum-induced DNA lesion (Wang et al., 2010). Intriguingly, in sharp contrast to 1,2-dGpG cisplatin-induced DNA lesions, the pyriplatin-DNA adduct can cross over the bridge helix and accommodate into the Pol II active site to support efficient CMP incorporation. Indeed, the correct CMP nucleotide can form a canonical Watson-Crick base pair with pyriplatin-modified guanosine (Figure 8b). The bulky pyridine ligand extrudes toward the major groove without disrupting the overall structure of the RNA/DNA duplex. Intriguingly, the platinum DNA adduct interacts with conserved bridge helix residues to stabilize the pre-translocation state and the presence of the bulky ligand also introduces a strong steric barrier for allowing the next template nucleobase to cross over the bridge helix to reach +1 template position during translocation. Therefore, the mechanism of transcriptional inhibition by pyriplatin differs significantly from that of cisplatin or UV damage (Wang et al., 2010).

The impacts of the phenanthriplatin-dG DNA lesion on transcription bypass and transcriptional fidelity were also systematically investigated recently (Kellinger et al., 2013). Strikingly, the presence of a phenanthriplatin-dG DNA lesion at +1 position does not affect the insertion step. The specificity for CMP incorporation is almost the same as for

undamaged dG on the template. Pol II maintains a very high discrimination power at the first checkpoint step even in the presence of the bulky phenanthriplatin-induced DNA lesion. In sharp contrast, the presence of the phenanthriplatin-induced DNA lesion causes Pol II to significantly slow down at the extension step and Pol II switches to an error-prone mode during lesion bypass. The presence of the phenanthriplatin-induced DNA lesion does not change proofreading activity in the third checkpoint step. The fact that Pol II can switch from an efficient error-free mode to a slow error-prone mode during lesion bypass somewhat resembles how DNA polymerases switch in translesion DNA synthesis (Friedberg, Lehmann, and Fuchs, 2005; Goodman, 2002). The difference is that translesion DNA synthesis requires enzyme switching between multiple DNA polymerases, whereas translesion RNA bypass requires only a single polymerase (Pol II) but at distinct modes (trigger loop dependent vs trigger loop independent mode).

Cyclopurine, such as 8,5'-cyclo-2'-deoxyadenosine (CydA), is a monofunctional bulky oxidative DNA lesion caused by ROS (Wang, 2008) that also strongly blocks RNA polymerase II and interferes with gene transcription in mammalian cells (Jaruga and Dizdaroglu, 2008). Pol II can also undergo slow bypass and generate both error-free and error-prone transcripts with AMP misincorporated immediately downstream from the lesion (Brooks et al., 2000; Marietta and Brooks, 2007; You et al., 2012). Recently, Kashlev and Wang lab presented biochemical and crystallographic evidences for the molecular mechanism of CydA recognition and lesion bypass by RNA Pol II (Figure 8c) (Walmacq et al., 2015). Interestingly, unlike pyriplatin or phenanthriplatin induced lesions, CydA-DNA lesions fail to support efficient UMP incorporation. In fact, the mode of CydA-DNA lesion bypass by Pol II is somewhat similar to that of CPD-lesions. The crystal structure of the Pol II complex containing a CydA lesion at the +1 register reveals a strikingly similar location of the DNA lesion in comparison with the CPD DNA lesion (Brueckner et al., 2007). Both lesions accommodate above the bridge helix. As a result, CydA lesion cannot support template-dependent UMP incorporation, rather preferential non-template dependent AMP incorporation is observed (A-rule). Similar to CPD lesions, Pol II also gets stalled due to the difficult loading of the template base (5') next to CydA into the active site.

3.4. Functional interplay between epigenetic DNA modifications and Pol II transcription

In addition to DNA lesions caused by endogenous or environment chemical agents, the DNA template also undergoes enzyme-catalyzed epigenetic DNA modification such as dynamic DNA methylation and demethylation, which are important in stem cell renewal and cell differentiation. During TET-mediated active DNA demethylation, 5-methylcytosine (5mC) can be oxidized to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) by TET enzyme in a step-wise manner (Branco, Ficz, and Reik, 2011; He et al., 2011; Ito et al., 2011; Munzel, Globisch, and Carell, 2011; Wu and Zhang, 2011). 5fC and 5caC can be further removed by thymine DNA glycosylase (TDG) in conjunction with base excision repair to regenerate unmodified cytosine. Emerging evidence suggests that these oxidized 5-methylcytosines (Oxi-mCs, such as 5hmC, 5fC and 5caC) may have critical impacts on transcription. Indeed, genome-wide mapping of these Oxi-mCs revealed specific enrichment at enhancers, promoters, and gene bodies (Shen et al., 2013; Song et al., 2013). A number of protein complexes involved in transcription, chromatin

remodelling, splicing, and DNA repair have been identified to selectively bind synthetic DNA containing Oxi-mCs (Hashimoto et al., 2014; Iurlaro et al., 2013; Spruijt et al., 2013).

It is interesting to note that the modifications of these Oxi-mCs are located at the 5-positions of cytosine facing toward major grooves, which do not interfere with Watson-Crick base pairing (Raiber et al., 2015). However, given the distinct chemical nature of the functional groups in these oxidized methylcytosines and potential to interact differently with protein residues, whether and how these modifications affect RNA Pol II transcription deserve exploration. To this end, the transcriptional effects of these oxidized intermediates at biochemical, structural and cellular levels were investigated recently (Kellinger et al., 2012; Wang et al., 2015; You et al., 2014). The *in vitro* transcription results using purified mammalian and yeast Pol II reveals transient pausing of Pol II at 5fC or 5caC site in comparison with other forms of cytosine (Kellinger et al., 2012). Further follow-up studies elucidated the molecular basis of Pol II retardation at 5fC/5caC sites. RNA Pol II can recognize 5caC through a specific interaction between its conserved epi-DNA recognition loop in the polymerase and the carboxyl group of 5caC (Figure 9a and 9b) (Wang et al., 2015). This hydrogen bonding interaction causes a positional shift for incoming GTP, thus compromising nucleotide addition. The global effect of increased 5fC/5caC levels on transcription was also determined, revealing that such DNA modifications indeed retarded Pol II elongation on gene bodies *in vivo*, which is consistent with previous *in vitro* observation. Modeling studies indicated that this epi-loop can also recognize 5fC but not 5hmC, 5mC or C during nucleotide incorporation (Figure 9c). These results demonstrate the functional impact between cytosine modifications and transcription activities and suggest a novel role for Pol II to function as a specific and direct epigenetic sensor during transcription elongation.

4. Conclusions

Recent studies combining structural biology, computational biology and synthetic chemical biology approaches provide different perspectives in understanding the molecular basis of transcriptional fidelity and functional interplay between DNA modifications and Pol II transcription. Structural biology reveals the snapshots of static atomic structures and detailed interaction network during Pol II transcription. Computational biology provides detailed dynamic information and conformational transitions of the Pol II transcription process. Synthetic chemical biology provides us with novel chemical tools to further dissect individual contributions of chemical interactions and nucleic acid structural motifs in controlling Pol II transcriptional fidelity. Taking all the evidence together, we now obtain a much more comprehensive understanding of the molecular mechanisms of Pol II transcriptional fidelity maintenance, which provides a framework to understand how DNA lesions and modifications affect Pol II transcriptional fidelity.

Recent mechanistic studies of the functional interplay between DNA lesions and modifications and Pol II transcription machinery greatly enhance our understanding of how Pol II deals with a variety of DNA modifications as it moves along the DNA template during transcription. It has now become clearer that in addition to the 'routine job' for RNA Pol II to synthesize a RNA transcript complementary to DNA template, the 'secret identity' of

RNA Pol II starts to appear. Indeed, it has been proposed that Pol II is a sensor for a variety of DNA lesions. Arrested Pol II at bulky DNA lesions triggers transcription-coupled repair, a specific DNA repair pathway, or ubiquitination (Bregeon et al., 2003; Burns et al., 2010; Cline et al., 2004; Dimitri et al., 2008; Dimitri et al., 2008; Dimitri et al., 2008; Donahue et al., 1996; Jung and Lippard, 2006; Kalogeraki, Tornaletti, and Hanawalt, 2003; Kuraoka et al., 2007; Lee et al., 2002; Mei Kwei et al., 2004; Monti et al., 2011; Neil, Belotserkovskii, and Hanawalt, 2012; Perlow and Broyde, 2003; Perlow et al., 2002; Schinecker et al., 2003; Tornaletti et al., 1997; Tornaletti, Maeda, and Hanawalt, 2006; Tornaletti et al., 2004; Tornaletti et al., 2003; Tornaletti, Reines, and Hanawalt, 1999; Wang et al., 2006). Recent research efforts also uncovered that Pol II can pause at natural unconventional DNA structures and epigenetic DNA modifications (Belotserkovskii et al., 2010; Belotserkovskii et al., 2013; Ditlevson et al., 2008; Krasilnikova et al., 2007; Salinas-Rios, Belotserkovskii, and Hanawalt, 2011; Tornaletti, 2009; Tornaletti, Park-Snyder, and Hanawalt, 2008). Taken together, Pol II may work as a scanner or integrator to scan the genome and sense a variety of DNA modifications and lesions, or unnatural DNA sequences or structures and triggers distinct downstream processes such as alternative splicing, chromatin remodelling, termination, and DNA repair. Further mechanistic studies using multidisciplinary approaches are needed to gain a comprehensive understanding of the functional interplay between DNA lesions/modifications and transcriptional machinery. This knowledge would also pave the way for developing novel therapies for transcription-related diseases in the future.

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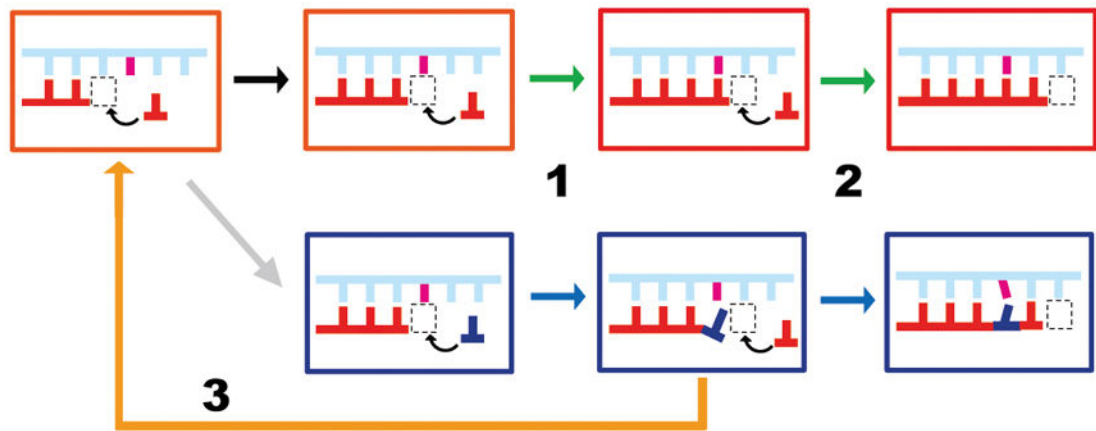


Figure 1.

Scheme of three fidelity checkpoint steps during Pol II transcription elongation. During the insertion step (step 1), the matched nucleotide (red) is selected over incorrect substrate (blue). During the extension step (step 2), the RNA transcript can be preferentially extended from a matched pair. During the proofreading step (step 3), cleavage of the RNA transcript containing a mismatched end is significantly more efficient than that for the fully matched end. The template DNA is shown in cyan and non-template DNA is omitted. The RNA primer is shown in red. The active site of Pol II is shown in the boxed area.

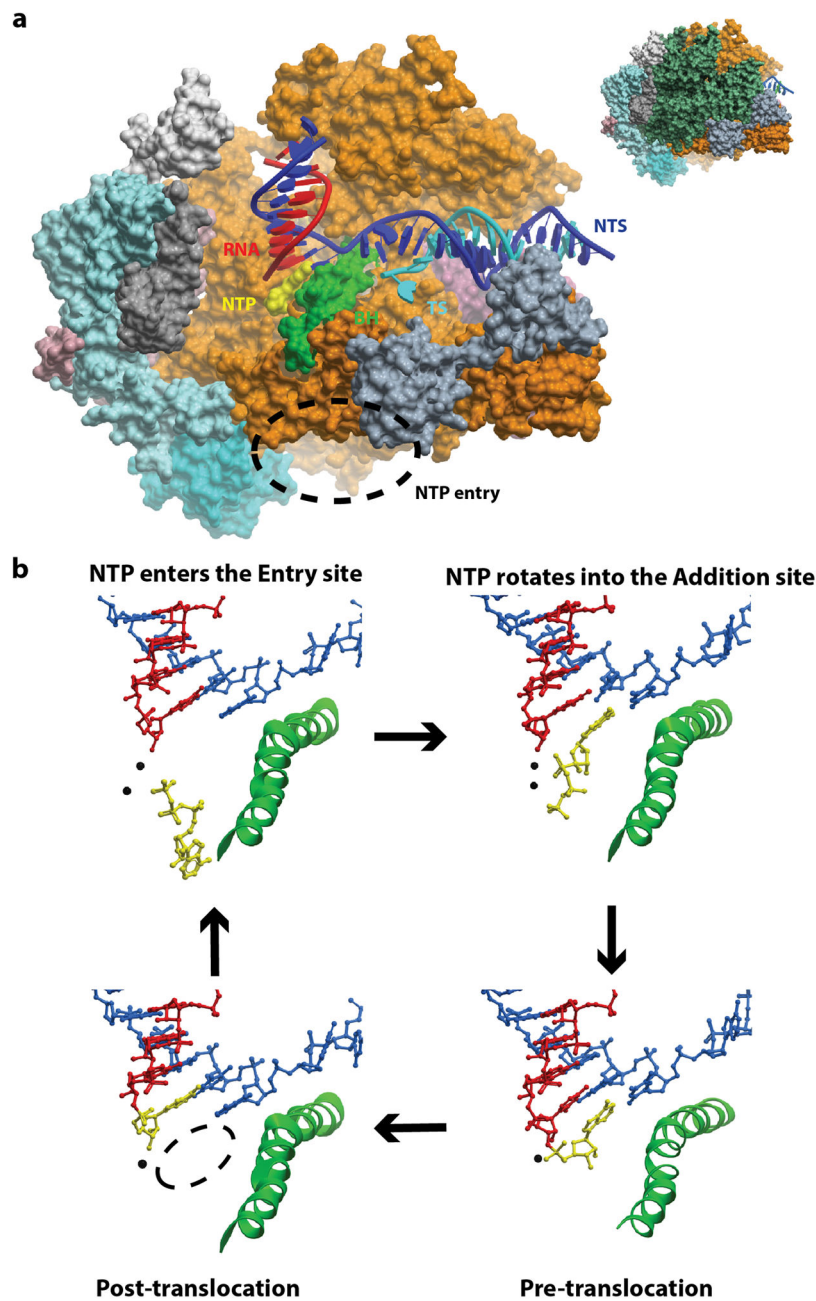


Figure 2. Structures of Pol II elongation complexes (EC) reveals key steps of the nucleotide addition cycle. (a) Overall architecture of RNA Pol II EC (PDB ID: 2E2H). Rbp2 is omitted here to reveal the position of RNA/DNA scaffold and active site. The incoming NTP enters Pol II active site through the secondary channel of Pol II at bottom (dashed circle). Bridge helix (BH) is shown in green. RNA, template DNA (TS), and non-template DNA (NTS) are shown in red, blue, and cyan, respectively (b) Nucleotide addition cycle during Pol II transcription elongation. The NTP can first bind to the “entry site”, followed by rotating into the “addition site” to form the matched base pair with the DNA template. After the catalysis,

Pol II translocate one base-step forward from a pre-translocation state to form a new post-translocation state, creating a new active site for the next round of nucleotide addition cycle.

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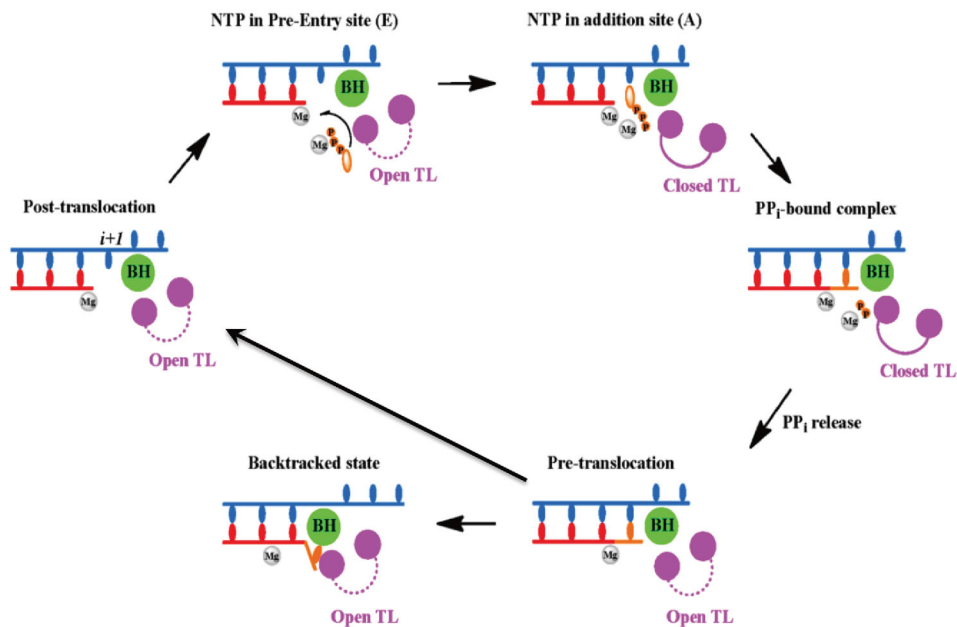
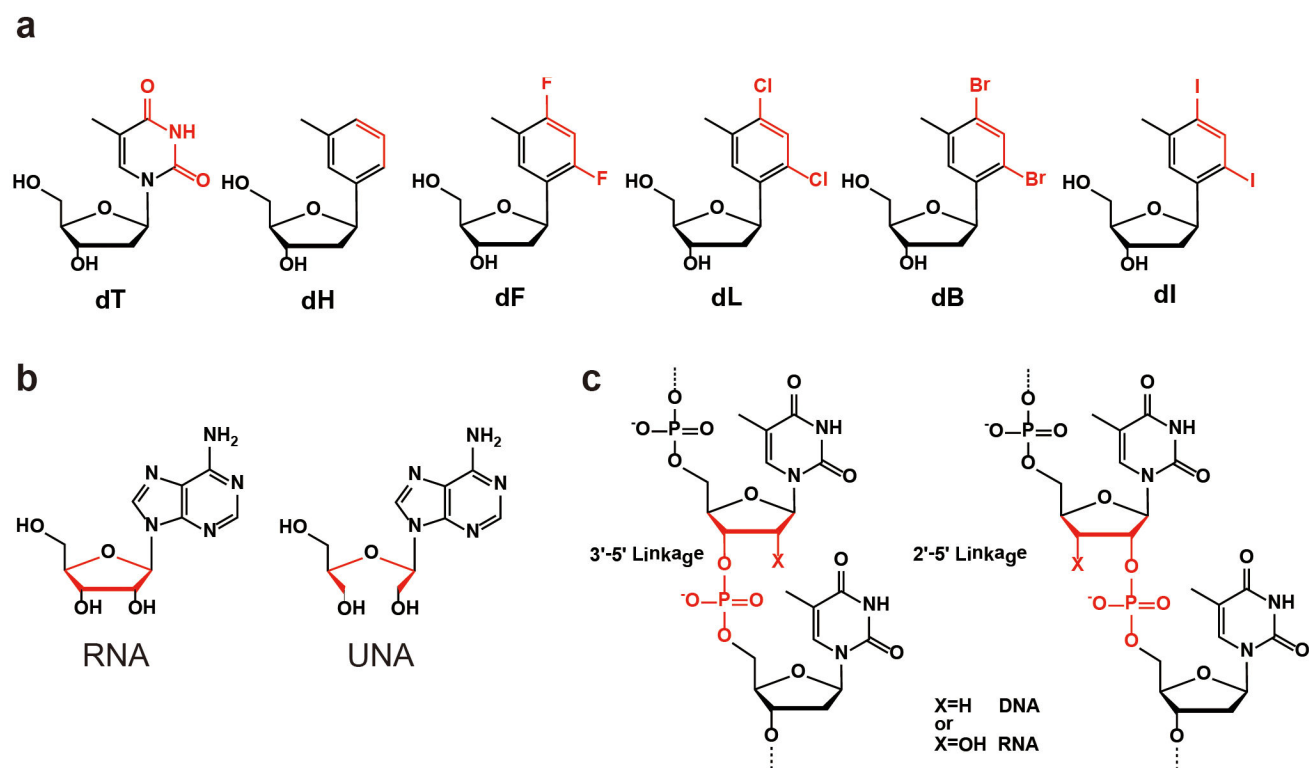


Figure 4. A complete nucleotide addition cycle depicted from both structural and computational evidence during the transcription elongation process. This process mainly consists of the following steps: (1) The NTP (in orange) binds to the post-translocation state of Pol EC, accompanied by the TL closing motion. (2) Catalytic reaction takes place and one pyrophosphate ion (PPi) is formed. (3) PPi releases from the active site, followed by the TL opening motion. (4) The pre-translocation state of Pol II EC translocates to the post-translocation state to reinitiate another NAC. (5) When misincorporation occurs, the Pol II EC can move in reverse direction to form a backtracked state. The NTP- and modeled PPi-bound Pol II EC complexes are shown in the inset where TL residues Leu1081 and His1085 are highlighted in cyan.

**Figure 5.**

Utilization of nucleotide analogues to dissect the individual contributions of chemical interactions and structural motifs to the transcriptional fidelity. (a) Chemical structures of several ‘hydrogen-bonding deficient’ thymidine (dT) analogues (dH, dF, dL, dB, dI) with sub-angstrom increments in size to probe the effect of hydrogen-bonding base pairing. (b) The unlocked nucleoside was designed to evaluate the roles of the sugar backbone in the transcriptional fidelity. (c) Phosphodiester linkage was mutated to elucidate the contribution of phosphodiester linkage orientation.

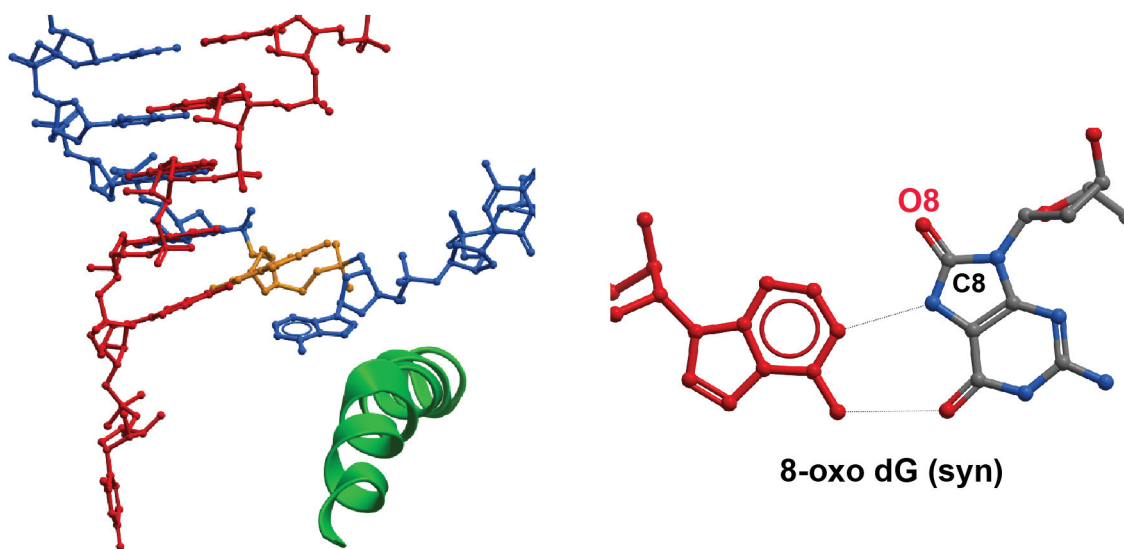


Figure 6. RNA Pol II recognition of 8-oxo dG lesion. 8-oxo dG adopts an a syn- form when pairing with adenine in the Pol II active site (PDB ID: 3I4N).

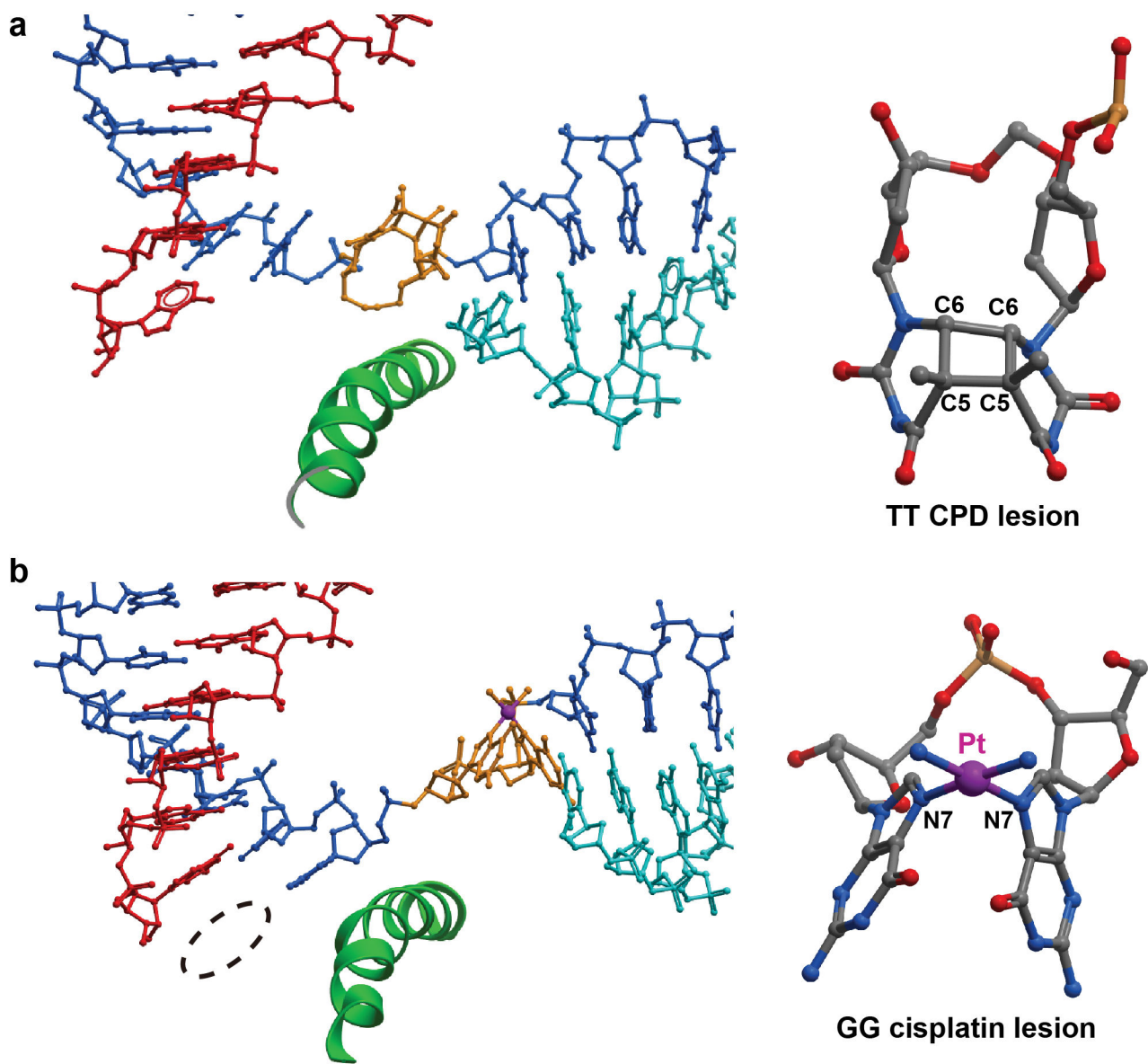


Figure 7. Structural basis of the 1,2-crosslinked DNA lesion-containing Pol II ECs. (a) The crystal structure of Pol II EC stalled at CPD lesion (PDB ID: 4A93). (b) The crystal structure of Pol II EC stalled by the 1,2-dGpG cisplatin DNA lesion (PDB ID: 2R7Z).

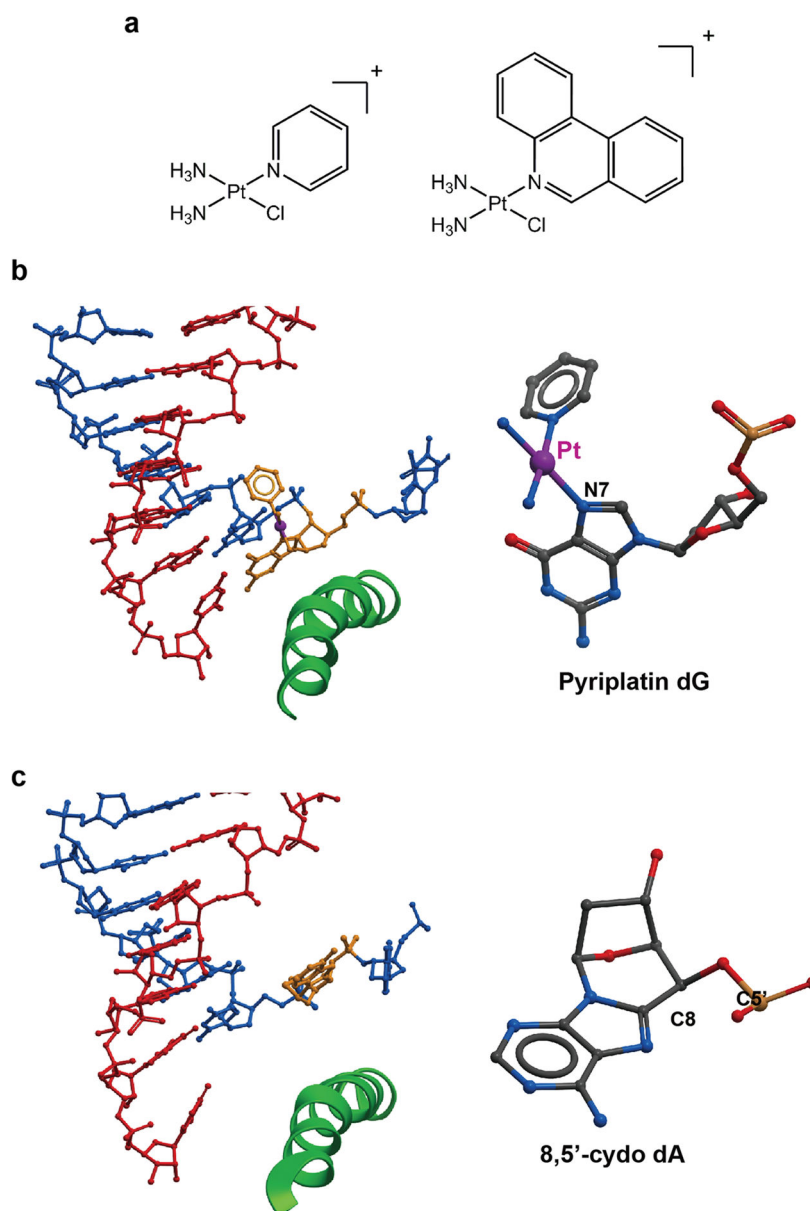


Figure 8. Recognition of the monofunctional bulky DNA lesion by Pol II EC. (a) Chemical structures of mono-functional platinum complexes, pyriplatin and phenanthriplatin. (b) The crystal structure of the Pol II EC stalled at a pyriplatin- DNA monofunctional adduct (PDB ID: 3M3Y). (c) RNA Pol II recognition of 8,5'-cydo dA (PDB ID: 4X6A). This bulky monofunctional oxidative damage fails to move over the bridge helix but stays in the “above-bridge-helix” conformation.

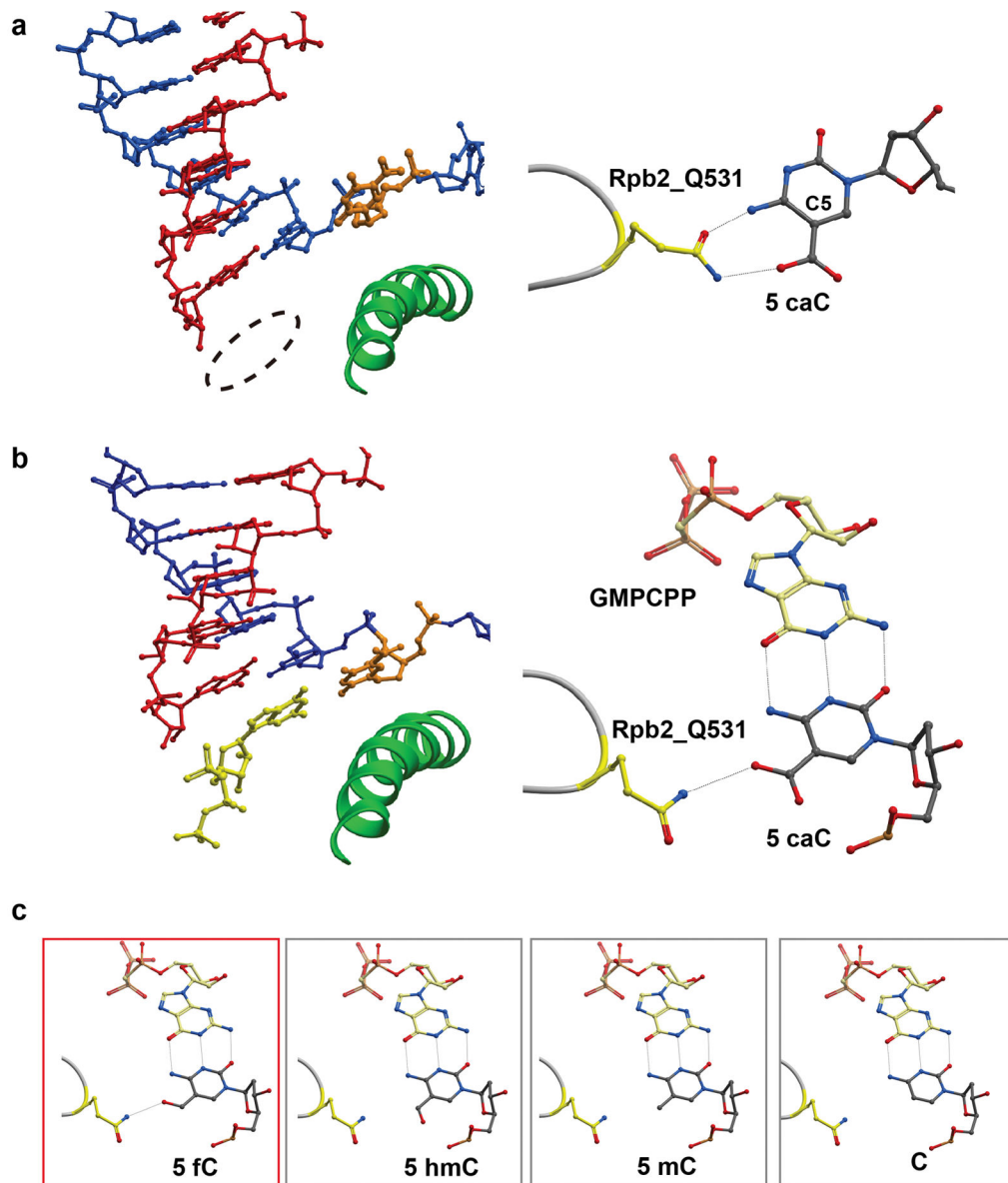


Figure 9.

Recognition of DNA demethylation intermediates by Pol II EC. (a) The crystal structure of the Pol II EC with 5cac, in which the key residue Q531 of Rpb2 subunit interacts with 5cac (PDB ID: 4Y52). About half of 5caC adopts the “above-bridge-helix” conformation. (b) Q531 can form hydrogen bond with 5caC results in a positional shift of the template away from the canonical position (PDB ID: 4Y7N). (c) Models of similar interaction between Q531 and 5fC template, but not for 5hmC, 5mC and C templates.

Note: The color version of figures are available online.