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# RNA polymerase fidelity and transcriptional proofreading

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Whereas mechanisms underlying the fidelity of DNA polymerases (DNAPs) have been investigated in detail, RNA polymerase (RNAP) fidelity mechanisms remained poorly understood. New functional and structural studies now suggest how RNAPs select the correct nucleoside triphosphate (NTP) substrate to prevent transcription errors, and how the enzymes detect and remove a misincorporated nucleotide during proofreading. Proofreading begins with fraying of the misincorporated nucleotide away from the DNA template, which pauses transcription. Subsequent backtracking of RNAP by one position enables nucleolytic cleavage of an RNA dinucleotide that contains the misincorporated nucleotide. Since cleavage occurs at the same active site that is used for polymerization, the RNAP proofreading mechanism differs from that used by DNAPs, which contain a distinct nuclease specific active site.

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## Introduction

To ensure genetic integrity, DNA polymerases (DNAPs) generally exhibit high fidelity, but low-fidelity DNAPs also exist that accomplish special tasks such as translesion synthesis (reviewed in [1–3]). RNA polymerases (RNAPs) also operate with high fidelity since transcription errors could result in non-functional non-coding RNAs or in erroneous mRNAs that could give rise to mutant proteins with impaired function [4]. Indeed transcription accuracy is relatively high, with an estimated error rate of less than $10^{-5}$ for bacterial and eukaryotic RNAPs [5–7]. Since DNAPs and RNAPs share the same catalytic mechanism, and since both enzyme families use a DNA template to produce a complementary nucleic acid strand, they may use similar mechanisms to ensure fidelity. Here, we first summarize the known mechanisms underlying DNAP fidelity, and then we describe our current understanding of RNAP fidelity mechanisms, which has improved considerably over the past few years owing to new functional and structural studies.

## DNAP fidelity mechanisms

The major contribution to DNAP fidelity is the high selectivity against incorporation of a wrong nucleotide, which is achieved to a large extent by the shape complementarity between a Watson–Crick base pair (bp) and the enzyme's active center [3,8,9]. Discrimination of mismatches from matched bps is achieved by recognition of the mismatch itself, rather than the surrounding sequence, and its efficiency depends on the type of mismatch [10–17]. DNAPs select dNTPs against rNTPs with a 'steric gate,' formed between two amino acid side chains of the active center that sandwich the substrate sugar moiety and exclude a 2′-OH group [18–23]. Important determinants for NTP selectivity are multiple contacts formed between DNAP and the minor groove of the template base-NTP bp [24].

DNAP fidelity further relies on mechanisms that act after nucleotide selection. In particular, DNAPs slow down or stop DNA extension after misincorporation and can cleave off a mismatched DNA product end with a 3′–5′ exonuclease activity, a reaction referred to as proofreading. In *E. coli*, the 3′–5′ exonuclease activity of DNAP I resides on a different domain than the polymerase activity [25–29]. DNAP III also has polymerase and exonuclease activities located at different active sites, but residing on separate subunits of the enzyme [30,31].

As shown for *Bacillus stearothermophilus* DNAP I, five sites on the enzyme are important for fidelity: (i) the insertion site, in which the NTP pairs with the template base, (ii) the catalytic site, in which the 3′-hydroxyl of the product strand and the two catalytic magnesium ions are located, (iii) the pre-insertion site, which houses the template base in a step before incorporation, (iv) the post-insertion site in which the growing 3′-end of the duplex DNA is located, and (v) the DNA template-product duplex binding region [32]. Structural studies of mismatch-containing DNAP–DNA complex structures have shown that mismatches can induce disruptions of the active site by (i) displacement of the template strand and disruption of the pre-insertion site; (ii) disruption of the product strand and the assembly of the catalytic site; (iii) disruption of both the template and product strands; and (iv) fraying of the DNA at the insertion site [32].

## RNAP fidelity mechanisms

Early work on RNAP fidelity [33,34] showed that misincorporation leads to slow addition of the next nucleotide, and that a mismatched RNA 3′-end can be removed with factors that stimulate the weak polymerase-intrinsic RNA 3′-cleavage activity. In a bacterial RNAP elongation complex (EC), a mismatched 3′-nucleotide induces an unactivated state of the enzyme, and is removed by Gre factors that stimulate RNA cleavage [33]. In human RNAP II, a mismatched 3′-nucleotide causes slow addition of the next nucleotide, and the intrinsic cleavage activity is stimulated by the factor TFIIS [34]. Thus, as in DNAPs, RNAPs achieve fidelity following two major strategies, substrate selection and proofreading, which involves the recognition and removal of a mismatched nucleotide although in contrast to DNAPs, the cleavage activity of RNAPs resides at the same, 'tunable' active center that carries out polymerization [35,36]. Our understanding of RNAP fidelity mechanisms was recently extended with biochemical and structural studies [37,38,39••,40•,41•,42••,43••,44•]. We describe below that these new studies show in more detail how transcription errors are prevented, recognized, and removed.

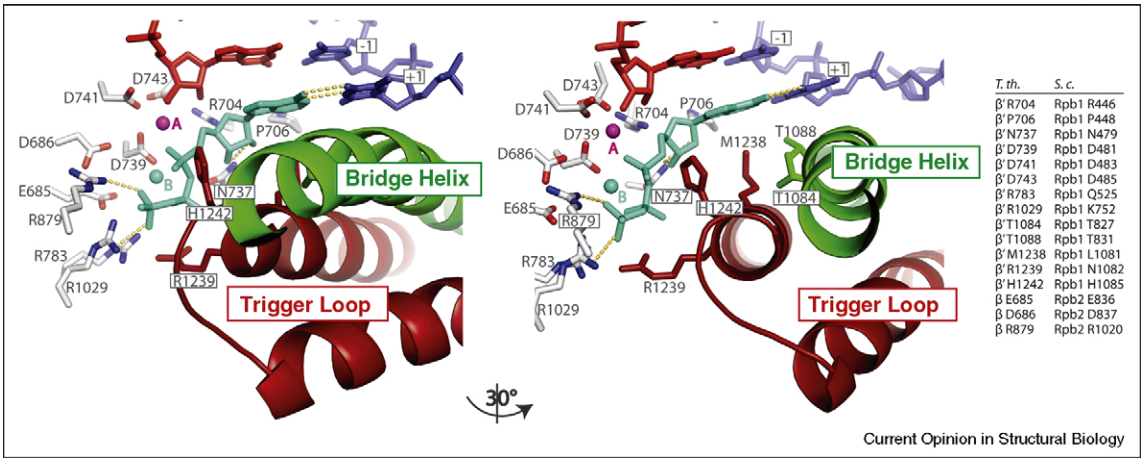
## Error prevention: RNAP selects the correct substrate

To select the correct NTP substrate, RNAPs discriminate rNTPs from dNTPs and select the rNTP that is complementary to the DNA template base. Substrate selection apparently occurs in two steps and involves isomerization of an open to a closed RNAP active center [41•,43••,45] (reviewed in [46,47]). The substrate first binds to an open active center in a pre-insertion state that probably allows sampling of NTPs. The correct NTP establishes Watson–Crick interactions with the DNA template and is then delivered to the insertion site [43••,48], where all contacts required for catalysis are formed (Figure 1). Closure of the active center involves folding of the mobile trigger loop [39••,41•,43••]. The single-subunit RNAP from phage T7 also uses a two-step mechanism of substrate loading [49,50], although in this enzyme two separate pre-insertion and insertion sites exist, whereas the NTP bound in the pre-insertion state of multisubunit RNAPs overlaps the NTP in the insertion site. A distinct pre-insertion site may exist for T7 RNAP since it does not have proofreading activity [51], and thus its fidelity relies entirely on the selection of the correct substrate.

There is evidence that the trigger loop and its surrounding residues are important for fidelity. Two mutations in the trigger loop of *E. coli* RNAP affect misincorporation [52]. Mutation of Rpb1 residue Glu1103 in the RNAP II trigger loop also promotes incorporation of incorrect substrates [41•]. Mutations of several other trigger loop residues have been shown to affect the fidelity of incorporation by RNAP II [44•]. Residues in the vicinity of the trigger loop also are important for fidelity, as shown for residue Asp675 of the second largest subunit β′ in bacterial RNAP [37]. Additionally, a non-complementary NTP shifts the equilibrium between closed and open conformations of the RNAP EC towards the open state, making release of the incorrect NTP likely and incorporation unlikely [41•].

To discriminate rNTPs from dNTPs, RNAPs recognize the 2′-OH group of the ribose. In *T. thermophilus* RNAP,

**Figure 1**




Error prevention: how RNAPs select the correct substrate. NTP (green cyan) bound to the insertion site of a bacterial RNAP elongation complex (PDB 2O5J [43••]). RNA is in red, DNA template strand in blue. The catalytic metal ions A and B are shown as a magenta and a green cyan sphere, respectively. Depicted in gray are side chains of β′ and β (corresponding to Rpb1 or Rpb2, respectively, in yeast RNAP II) at a distance of up to 5 Å around the NTP. *Thermus thermophilus* (*T.th.*) RNAP residues and their corresponding residues in yeast RNAP II (*S.c.*) are provided in a table [43••]. Hydrogen bonds between the NTP and RNAP are indicated by yellow dashed lines. To illustrate the NTP insertion site, we used here the bacterial NTP complex structure rather than the yeast core RNAP II NTP complex since it contains an intact RNA 3′-hydroxyl group.

the O2′ and O3′ of the rNTP ribose form hydrogen bonds with Asn737 of the largest RNAP subunit β′ [38,43••] (Figure 1). Mutation of the corresponding β′ Asn458 of the *E. coli* enzyme leads to a dramatic increase of incorrect dNTP incorporation [38]. In eukaryotic RNAP II, the corresponding residue Asn479 of subunit Rpb1 was also proposed to be involved in the discrimination of the ribose sugar by interaction with the 2′-OH of the incoming rNTP [45,53] or with its O3′ atom [39••]. Mutation of Asn479 in the RNAP II system to serine led to decreased rNTP versus dNTP discrimination similar to the bacterial system, but this effect was apparently due to loss of interaction with the 3′-OH group [39••]. It has recently been shown that also Rpb1 residue His1085 is crucial for the selection of the correct sugar of the substrate [44•]. In T7 RNAP, the hydroxyl group of Tyr639 forms a hydrogen bond with the 2′-OH of an incoming rNTP [54–56].

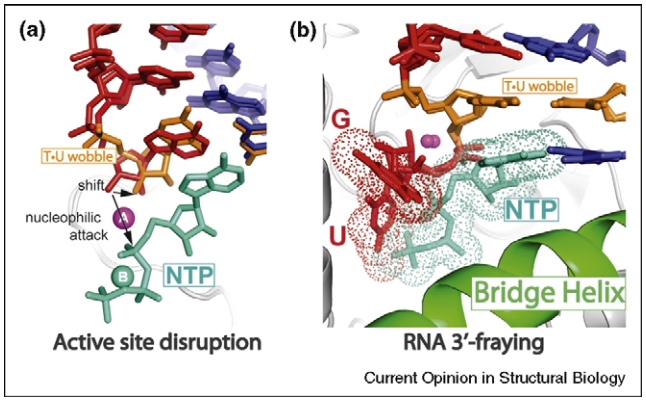
Despite these mechanisms to ensure incorporation of the correct substrate, misincorporations do occur. Systematic studies of RNAP II showed that misincorporation efficiency depends on the type of mismatch [42••]. The different misincorporation efficiencies are broadly consistent among different RNAP II studies [34,41•,42••] but differ strongly from those reported for DNAPs [10–17], probably reflecting a different active center structure in both types of enzymes. In RNAP III, mismatch-specific misincorporations were also observed [40•]. Misincorporation may result from mispairing of the substrate with the template [42••] but also from non-templated nucleotide incorporation since RNAP II can misincorporate opposite an abasic template site [57]. In addition, misincorporation can result from transcription of DNA lesions [57,58] or by template misalignment [59•,60].

## Error detection: mismatches induce off-line states

Once a misincorporation error has occurred, RNAPs must detect the mismatch and slow down transcription to open a time window for proofreading. In RNAP III, misincorporation led to a slow-down of RNA extension [40•]. A systematic study of RNA extension efficiencies by RNAP II revealed that extension is always slower after misincorporation, but that the efficiencies vary with the type of mismatch [42••]. RNA extension is particularly impaired for those mismatches that are efficiently formed [42••]. Thus, RNAP avoids to make mistakes twice; if it misincorporates, it does not extend the mismatch, and it prevents formation of mismatches that would be easily extended.

There is evidence that mismatches impair RNA extension by inducing off-line states of the EC. A mismatch can stably bind to RNAP II and disrupt the catalytically competent active site conformation. For example, a T·U mismatch at the −1 position (+1 defines the nucleotide addition site, −1 refers to the first upstream position)

**Figure 2**



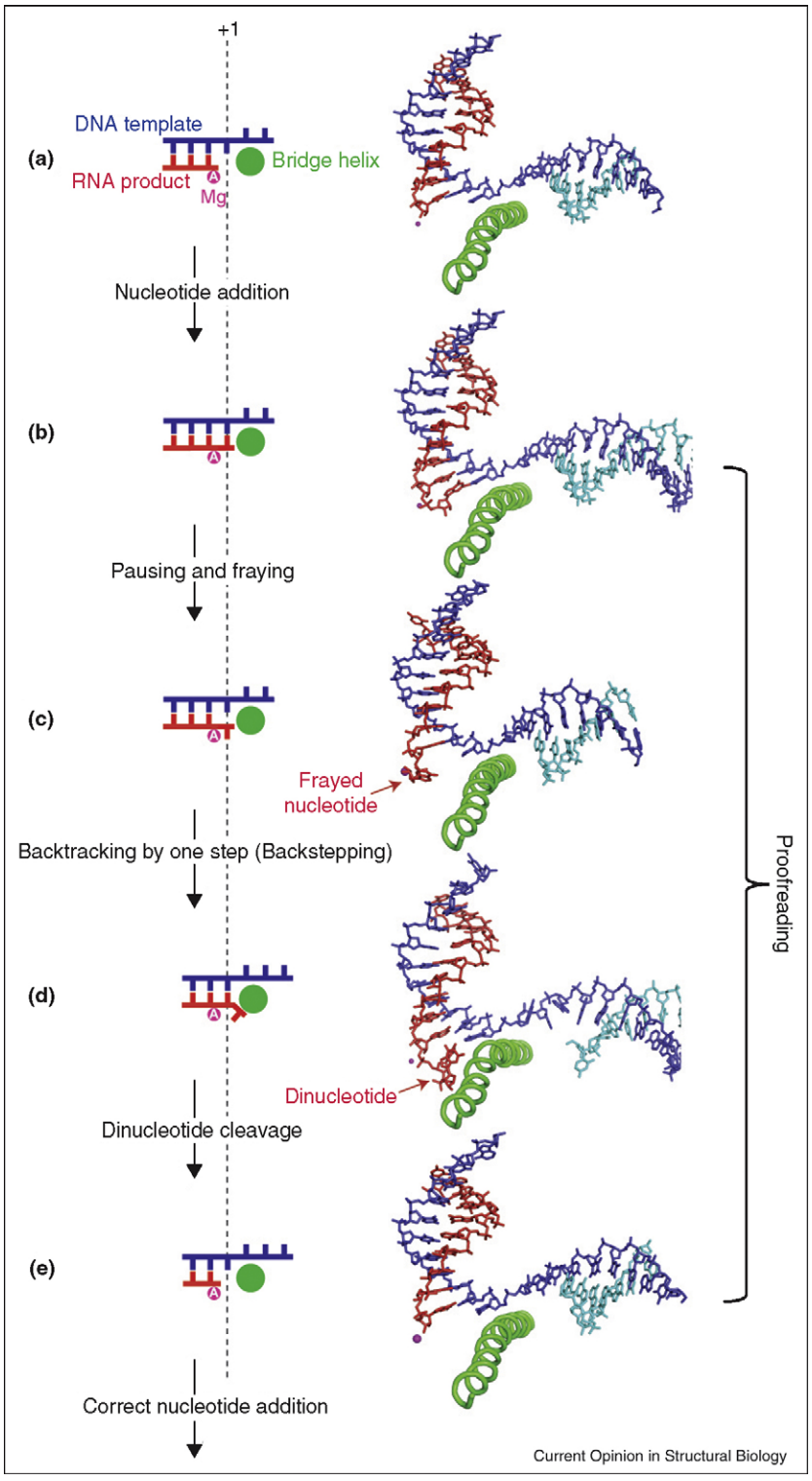

Error detection: how mismatches induce off-line states. **(a)** An example for active site disruption after misincorporation. Superposition of a mismatched RNAP II EC containing a T·U wobble pair (orange, PDB 3HOU) with a corresponding matched EC (PDB 3HOV) reveals a ~2 Å shift of the RNA 3′-hydroxyl (horizontal arrow). As a consequence, the RNA 3′-end is no longer in a position suited for an in-line nucleophilic attack (vertical arrow) of the phosphodiester bond between the α and β phosphates of the incoming NTP substrate (green cyan, taken from PDB 2O5J [43••]). The mismatched wobble pair is in orange. **(b)** RNA 3′-fraying pauses transcription. Frayed RNA 3′-nucleotides as observed in RNAP II ECs (PDB 3HOW and 3HOZ) clash with an NTP bound to the insertion site (green cyan, taken from PDB 2O5J [43••]). Van der Waals radii are illustrated by colored dots. Figure adapted from [42,84••].

can cause loss of the catalytic metal ion A and misalignment of the nucleophilic RNA 3′OH group [42••] (Figure 2a). A mismatch can also induce an unactivated state [33] that probably corresponds to the elemental paused state of RNAP, a common intermediate during elongation that results from a rearrangement of the EC inhibiting nucleotide addition without backtracking [61–63,64•,65]. Site-directed crosslinking and mutagenesis revealed that this elemental pause contains a frayed RNA 3′-terminal nucleotide [63,64•,66]. Structural studies have now revealed two different locations for a frayed nucleotide in the pore beneath the RNAP active site [42••]. The fraying sites both overlap the NTP-binding site, explaining how they pause nucleotide addition [42••] (Figure 2b). In one site (fraying site I), the nucleotide binds parallel to the axis of the DNA–RNA hybrid, whereas it binds perpendicular to it in another (fraying site II).

## Error removal: RNAP backtracking and RNA cleavage

Multisubunit RNAPs possess an intrinsic nucleolytic RNA cleavage activity. RNA cleavage leads to a new 3′-OH group at the RNA end at the polymerase active site, allowing RNA synthesis to resume [67–69]. RNAPs I and III possess a strong intrinsic RNA cleavage activity [70,71•]. In RNAP III, cleavage activity is so strong that misincorporation cannot be detected unless a cleavage-deficient isoform of the enzyme is used [40•]. RNA

**Figure 3**




Error removal: model of the RNAP proofreading cycle. Crystal structures of RNAP II ECs in different functional states suggest a model for transcriptional proofreading. The vertical dashed line indicates register +1, the nucleotide addition site. **(a)** post-translocation state (PDB 1Y1W), **(b)** pre-translocation state (PDB 1I6H, downstream DNA was modeled from 1Y1W [47], **(c)** paused state with a frayed 3′-RNA guanine (PDB 3HOW), **(d)** backtracked state (PDB 3GTJ), **(e)** post-translocation state. In this structure, dinucleotide cleavage occurred after the crystallization setup (PDB 3HOY).

cleavage occurs in dinucleotide steps in bacterial, eukaryotic and archaeal RNAPs [34,68,72–75]. For dinucleotide cleavage to occur, the scissile phosphodiester bond must be aligned with the catalytic site, which is achieved by RNAP backtracking. During backtracking, the terminal, mismatched nucleotide is moved from position +1 to position +2, the first position downstream of the nucleotide addition site. Recent structural studies revealed RNA backtracked by one position and suggested the RNA end to occupy a site from which cleavage of a dinucleotide can occur (referred to as proofreading site) [39••]. This site overlaps with the fraying site II.

## Model for transcriptional proofreading

The recent structural studies [42••,84••] together with published biochemical work [34,40•,85•] converge on a model for the mechanism of RNAP proofreading (Figure 3). After misincorporation, the mismatched nucleotide at position +1 of the RNA is frayed away from the template, thereby pausing RNAP. Pausing is the first step in backtracking [63,76–83]. The frayed nucleotide then inhibits RNA extension, because it prevents NTP binding, but favors backtracking, because the bp in position +1 is disrupted. RNAP then backtracks by one position and slightly shifts the mismatched RNA nucleotide from a fraying site to an overlapping proofreading site. Subsequent dinucleotide cleavage results in a new RNA 3′-OH group and an empty NTP-binding site, thus re-accessing an on-line state that allows transcription to resume. This mechanism is consistent with the observation in DNAPs that the 3′-cleavage rate is governed by the rate of fraying [86].

RNAP II fidelity is increased *in vitro* by preferential removal of mismatched RNA ends in the presence of TFIIS [34,41•,57,84••,87,88], although *in vivo* fidelity does not entirely depend on TFIIS, but more on the RNAP II subunit Rpb9, emphasizing the importance of the RNAP intrinsic cleavage activity [89–91]. Preferential removal of misincorporated RNA residues has also been observed in *E. coli*, increased by cleavage-stimulatory Gre factors [33] and in the archaeal system that contains the cleavage factor TFS [75,92]. Cleavage stimulatory factors may recruit the second metal ion required for catalysis or the hydrolytic water molecule. They also may position the substrates or backtracked RNA, or induce re-mobilization of backtracked RNA bound to non-productive sites. The structures of RNAP II ECs with TFIIS do not reveal the course of backtracked RNA around the active center [45,84••], and more studies are therefore required to determine in detail how cleavage stimulatory factors work.

## Conclusions

Recent functional and structural studies of RNAP fidelity mechanisms revealed similarities and differences to DNAP fidelity mechanisms. Fidelity is achieved by two strategies, selection of the correct NTP substrate, and proofreading, which involves detection and removal of the misincorporated nucleotide. Selection of the correct NTP is probably governed by similar mechanisms in DNAPs and RNAPs, and involves an induced fit mechanism that requires closure of the active center induced by an accurate base pairing of the NTP with the template base. However, the mechanism of proofreading is entirely different in DNAPs and RNAPs. Whereas an erroneous DNA end is transferred from the DNAP polymerization site to a DNA nuclease active site that resides in a different domain or subunit, an erroneous RNA end remains at the same, single 'tunable' active site of RNAP that can switch from polymerization mode to nuclease mode. A model for how proofreading by RNAP is accomplished has emerged and can be tested in detail in the future.

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