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Unlocking the Sugar "Steric Gate" of DNA Polymerases†

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ABSTRACT: To maintain genomic stability, ribonucleotide incorporation during DNA synthesis is controlled predominantly at the DNA polymerase level. A steric clash between the 2′-hydroxyl of an incoming ribonucleotide and a bulky active site residue, known as the "steric gate", establishes an effective mechanism for most DNA polymerases to selectively insert deoxyribonucleotides. Recent kinetic, structural, and in vivo studies have illuminated novel features about ribonucleotide exclusion and the mechanistic consequences of ribonucleotide misincorporation on downstream events, such as the bypass of a ribonucleotide in a DNA template and the subsequent extension of the DNA lesion bypass product. These important findings are summarized in this review.

On the basis of primary sequence similarity, DNA polymerases (Pols)[1] are classified into one of six families: A, B, C, D, X, or Y (*1*−*3*). In addition, the reverse transcriptase (RT) family is sometimes considered to be an additional family because each RT possesses both DNA- and RNA-dependent DNA polymerase activities. DNA polymerases catalyze nucleotidyl transfer of the four natural deoxyribonucleotide 5′-triphosphates (dNTPs) during DNA replication, repair, lesion bypass, sister chromatid exchange, and antibody generation processes (*4*). Despite functioning in diverse cellular roles, all in vitro characterized DNA polymerases catalyze nucleotide incorporation using a two-divalent metal ion mechanism, follow a minimal kinetic pathway, and share a similar structural architecture of the polymerase domain that is composed of the fingers, palm, and thumb subdomains (*5*, *6*). These subdomains move in response to the binding of an incoming dNTP and are thought to contribute to polymerase fidelity because of different conformational dynamics induced by correct and incorrect dNTPs (*7*−*11*). In addition, the fidelity of nucleotide incorporation is achieved mostly during two general steps of the polymerase kinetic pathway: nucleotide binding and nucleotide incorporation.[1]

Throughout most of the cell cycle, the intracellular concentration of ribonucleotide 5′-triphosphates (rNTPs) exceeds the levels of dNTPs by 10−2000-fold depending on the rNTP and dNTP pair and organism (*12*−*14*). Therefore, DNA polymerases have evolved mechanisms for restricting the misincorporation of rNTPs, substrates with an incorrect sugar, during DNA synthesis. Recently, it has been reported that *Saccharomyces cerevisiae* replicative DNA polymerases α, δ, and ε may misincorporate more than 10000 rNTPs during a single round of nuclear genomic replication in yeast (*14*, *15*). This result suggests that rNTPs may be the most common aberrant nucleotides inserted into a eukaryotic genome. In addition, following misincorporation during gap-filling DNA synthesis, rNTPs can become embedded into genomic DNA during the final ligation step in DNA repair pathways (*16*−*18*). Together, these studies highlight the biological importance of understanding the mechanisms of sugar selection employed by DNA polymerases that are the primary deterrents of rNTP incorporation into genomic DNA. This review will focus on the kinetic and structural basis of how a DNA polymerase discriminates between dNTPs and rNTPs during the incorporation, extension, and bypass processes.

MOLECULAR BASIS OF SUGAR SELECTION

Expanded Steric Exclusion Model: Either an Active Site Residue's Side Chain or a Protein Backbone Segment Plays the Major "Steric Gate" Role in Steric Discrimination against rNTPs. Most DNA polymerases and RTs have evolved a stringent nucleotide selection mechanism for preventing misincorporation of rNTPs into DNA (*19*). The rejection of rNTPs occurs via a simple steric exclusion model: an active site residue, usually one with a bulky side chain, collides with the 2′-OH group on the ribose ring of an incoming rNTP (Figure 1). To date, the amino acid residues, or steric gates, involved in regulating sugar discrimination include Glu for A family polymerases (*20*, *21*) and Tyr or Phe for members of the B, X, Y, and RT families (*21*−*31*). From the aforementioned steric clash, this mechanism of discrimination between dNTPs and rNTPs has sugar selectivity values, defined as dNTP incorporation efficiency divided by rNTP incorporation efficiency, measured to be 280−4400000 using kinetic and quantitative gel-based techniques for most DNA polymerases and RTs (Table 1). The kinetic basis of

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[1]Abbreviations: Dbh, DinB homologue of *Sulfolobus acidocaldarius*; ddNTP, 2′,3′-dideoxyribonucleoside 5′-triphosphate; dNTP, 2′-deoxyribonucleoside 5′-triphosphate; Dpo4, DNA polymerase IV of *Sulfolobus solfataricus*; FEN1, flap endonuclease 1; HIV-1, human immunodeficiency virus type I; KF, Klenow fragment of *Escherichia coli* DNA polymerase I; MMLV, Moloney murine leukemia virus; Pol, DNA polymerase; rNMP, ribonucleoside 5′-monophosphate; rNTP, ribonucleoside 5′-triphosphate; RT, reverse transcriptase; *Taq*, *Thermus aquaticus* DNA polymerase I; TdT, terminal deoxynucleotidyl transferase; WT, wild type.

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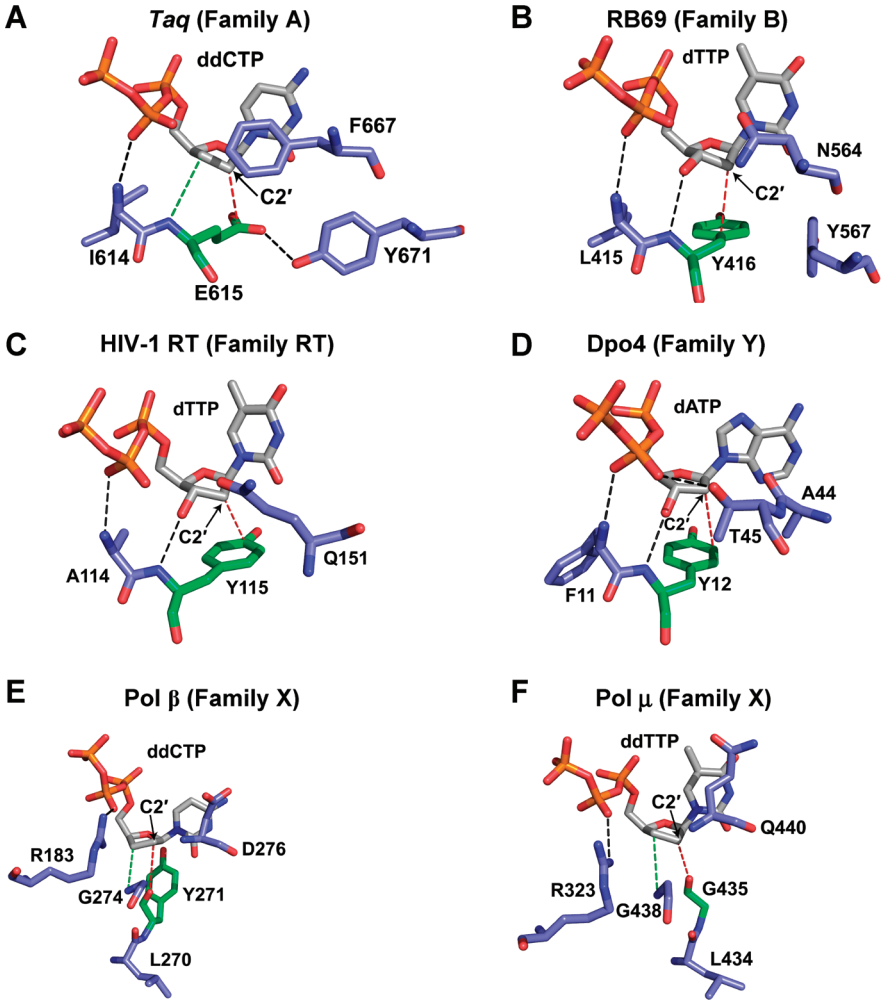

FIGURE 1: Structural basis for ribonucleotide exclusion. The steric gate residues (green sticks) and other nearby active site residues (lavendar sticks) are shown with the incoming nucleotide (gray sticks) for (A) *Taq* (PDB entry 3KTQ), (B) RB69 (PDB entry 1IG9), (C) HIV-1 RT (PDB entry 1RTD), (D) Dpo4 (PDB entry 2AGQ), (E) human Pol β (PDB entry 1BPY), and (F) mouse Pol μ (PDB entry 2IHM). The C2′ position is indicated using an arrow for each nucleotide. The shortest distance between the steric gate and C2′ position is indicated in each case with a red dashed line. Hydrogen bonding interactions are shown as black dashed lines, while potential hydrogen bonds with the missing 3′-OH group are shown as green dashed lines.

inefficient rNTP incorporation is due to the weaker binding and slower rate of incorporation compared to those of dNTPs (*20*, *26*, *28*, *29*, *31*, *32*). In general, the incorporation step is affected more than the binding step on the basis of the pre-steady state kinetic parameters for insertion of a dNTP versus rNTP. However, rat Pol β, the *Sulfolobus acidocaldarius* DinB homologue (Dbh), and human Rev1 do not discriminate during the nucleotide binding step, thereby suggesting the active site is not optimally assembled for the steric gate residue to clash with the 2′-OH group (*28*, *32*, *33*). Reducing the size of the side chain at the steric gate position (i.e., from E/Y/F to V/A/G mutation) creates a DNA polymerase that incorporates matched rNTPs as efficiently as correct dNTPs, thereby leading to relatively low sugar selectivity values of 2−33 (Figure 2) (*20*, *24*−*26*, *28*). Thus, the side chain of a steric gate residue is closest to the C2′ position, which would clash with a 2′-OH group of an incoming rNTP (Figures 1 and 3). This putative steric clash is modeled using the Y12 residue of DNA polymerase IV (Dpo4) and an incoming rATP (Figure 3B). Here, the Y12 residue as well as the triphosphate and adenine base of an incoming dATP were unaltered [Protein Data Bank (PDB) entry 2AGQ], while the ribose ring of rATP bound to N5-CAIR synthetase (PDB entry 3ETH) was used to replace the ribose of the incoming dATP in the Dpo4 crystal structure (*34*, *35*). Using SwissPDB Viewer (*36*), Tyr12 was mutated to alanine; this smaller side chain creates additional space in the binding pocket for a 2′-OH group (Figure 3C). These modeling results are supported by our pre-steady state kinetic analysis of the Dpo4 Y12A mutant (*37*) and are consistent with the ternary crystal structures of the same mutant in complex with DNA and an incoming dNTP or rNTP (K. Kirouac, Z. Suo, and H. Ling, unpublished data).

However, the classical steric gate model may not apply to every DNA polymerase. For example, crystallographic evidence suggested that X family DNA polymerases β (*38*, *39*) and λ (*40*) use a protein backbone segment, rather than a large side chain discussed above, to exclude rNTPs (Figure 1E). This prediction was tested recently for human Pol λ (*31*). Using site-directed mutagenesis and pre-steady state kinetic techniques, it was shown that the side chain of Y505 plays a minor role in sugar selection, thereby suggesting that the backbone segment of Y505−G508 likely governs ribonucleotide exclusion in Pol λ's active site (Figure 2). In contrast, Pol μ and terminal deoxynucleotidyl transferase (TdT), two members of the X family, lack a proficient mechanism for rNTP discrimination because of a Gly residue that is encoded at the putative steric gate position (Figure 1F) (*16*, *41*, *42*). Replacing G433 with Tyr in human Pol μ enhanced

Table 1: Measurements of Sugar Selectivity Values for Various DNA Polymerases and RTs

polymerase family	DNA polymerase or RT	sugar selectivity[a]	method	ref
A	*Escherichia coli* KF (E710)	3100−1700000	pre-steady state and steady state	*20*, *21*
	Taq (E615)	82000−1600000	steady state	*21*, *55*
	human Pol γ (E895)	1000	steady state	*66*
B	ϕ29 DNA polymerase (Y254)	4400000	pre-steady state	*24*
	RB69 (Y416)	64000	pre-steady state	*26*
	Vent (Y412)	2000−10000	steady state and pre-steady state	*21*, *29*
	bovine Pol α (Y865)	20000	steady state	*67*
	human Pol α (Y865)	500	steady state	*66*
	yeast Pol δ (Y613)	13000−1700000	primer extension and steady state	*14*
X	rat Pol β (Y271)	2000−6000	steady state and pre-steady state	*16*, *32*
	human Pol β (Y271)	8000	steady state	*68*
	human Pol λ (Y505)	3000−50000	pre-steady state	*31*
	human Pol μ (G433)	1.3−11	steady state and primer extension	*16*, *42*
	human TdT (G448)	2.6−8.9	steady state	*16*
	mouse TdT (G449)	2.0−4.9	steady state and primer extension	*41*
Y	Dbh (F12)	3400	pre-steady state	*26*
	Dpo4 (Y12)	5500−20500	pre-steady state	*37*
	human Rev1 (F428)	280	pre-steady state	*33*
RT	HIV-1 RT (Y115)	42000−130000	steady state	*25*, *57*
	MMLV RT (F155)	1100−21000	steady state	*21*, *22*

[a]Calculated as dNTP incorporation/rNTP incorporation.

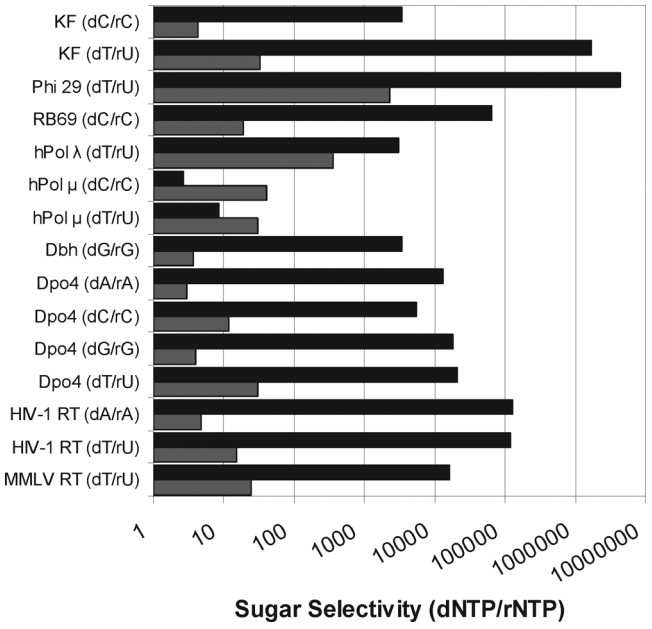

FIGURE 2: Measurements of sugar selectivity for steric gate mutants. The sugar selectivity values of wild-type (WT) (black bars) and mutant (gray bars) DNA polymerases and RTs were obtained from the references listed in Table 1. The following mutations were used: E710A for KF, Y254V for ϕ29, Y416A for RB69, Y505A for hPol λ, G433Y for hPol μ, F12A for Dbh, Y12A for Dpo4, Y115A for HIV-1 RT, and F155V for MMLV RT. The incoming dNTP and rNTP pairs are given in parentheses.

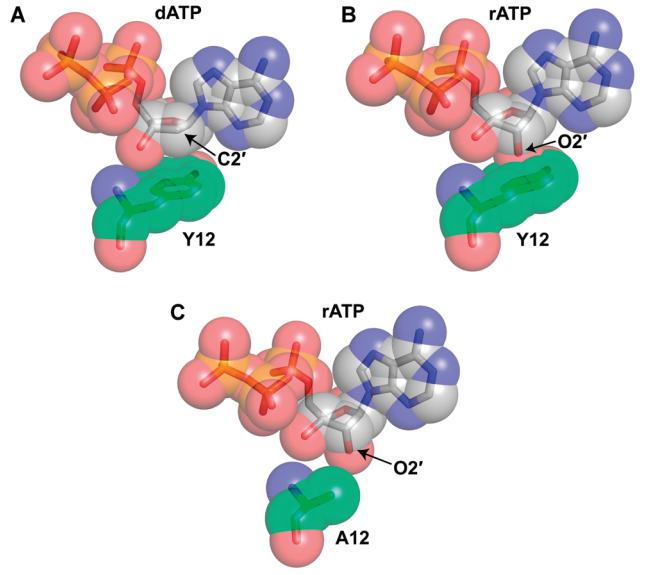

FIGURE 3: Structural models of a ribonucleotide in the active site of Dpo4. The steric gate residue (green sticks) and incoming nucleotide (gray sticks) are shown for (A) WT Dpo4 with dATP (PDB entry 2AGQ), (B) WT Dpo4 with rATP, and (C) Dpo4 Y12A with rATP. The ribose ring of rATP (B and C) is from PDB entry 3ETH, while the side chain of Y12 is from PDB entry 2AGQ and was replaced with Ala in panel C using SwissPDB Viewer (*36*). The 2′ position is denoted with an arrow.

the sugar selectivity value by at least 3−19-fold depending on the dNTP and rNTP pair (Figure 2) (*42*). Taken together, either a steric gate residue or a protein backbone segment plays a major role in the steric exclusion of rNTPs by a DNA polymerase or RT, which has led to an expansion of the classical steric exclusion model.

Replicative DNA polymerases usually possess 3′ → 5′ exonuclease proofreading activity; therefore, misincorporated rNMPs are potential substrates for this editing function. Studies based on wild-type (WT) ϕ29, Klenow fragment (KF) of *E. coli* DNA polymerase I, T4 DNA polymerase, and T7 DNA polymerase show that incorporated rNMPs are excised with efficiencies similar to those of complementary dNMPs (*24*, *43*). However, the extension of an rNMP-terminated primer by the polymerase active site of a replicative DNA polymerase is usually slow, which facilitates the excision of the rNMP moiety by the 3′ → 5′ exonuclease activity through two competing kinetic pathways: (i) direct transfer of the primer strand from the polymerase active site to the exonuclease active site for editing and (ii) first dissociation of DNA from the polymerase active site and then the rebinding of DNA at the exonuclease active site for editing. An improved understanding

of how misincorporated rNMPs affect the kinetic partitioning between the polymerase and exonuclease active sites is needed, especially when yeast replicative Pols α, δ, and ε may incorporate 10000 rNTPs during genomic replication (*14*, *15*). Overall, nucleotide discrimination at the polymerase active site is likely more important for preventing rNTP insertion than removal by the exonuclease active site. In general, both high- and low-fidelity DNA polymerases achieve overlapping sugar selectivity values, thereby reflecting the fact that the universal mechanism of steric exclusion is conserved among the polymerase families (Table 1). However, the low-fidelity DNA polymerases (e.g., X and Y family members in Table 1) are less likely to effectively distinguish dNTPs from rNTPs than the high-fidelity polymerases (e.g., A and B family members in Table 1) (*44*). For example, the range of sugar selectivity values is 500−4400000 for the high-fidelity polymerases compared to 1.3−50000 for the low-fidelity polymerases (Table 1). Such differences in nucleotide specificities between high- and low-fidelity DNA polymerases may be related to the overall flexibility and arrangement of their distinct active sites (Figure 1) (*44*−*48*).

Role of the Residues Flanking the Steric Gate Residue on rNTP Exclusion. The nucleotide binding pocket is defined by specific interactions between the active site residues and the nucleotide substrate. In addition to the side chain of a steric gate residue, the backbone NH group of the steric gate residue or a glycine for the X family members is within hydrogen bonding distance of the 3′-OH group of an incoming nucleotide. Other active site residues are necessary to maintain the proper orientation of the nucleotide and the side chain of the steric gate residue to achieve high sugar selectivity. For example, a highly conserved hydrophobic amino acid residue [e.g., I614 for *Thermus aquaticus* DNA polymerase I (*Taq*), L415 for RB69, A114 for HIV-1 RT, etc.], which flanks the N-terminus of the steric gate residue, can form a hydrogen bond between its backbone NH group and a nonbridging oxygen in the β-phosphate of a bound nucleotide for most of the polymerase families. For the A family member *Taq*, the nucleobase is stacked and stabilized by the side chain of F667 while the side chain hydroxyl group of Y671 is hydrogen bonded to the steric gate E615 (Figure 1A). Meanwhile, the Y family member Dpo4 uses A44 to stack with the base of the incoming nucleotide and the side chain hydroxyl group of the adjacent residue T45 to form a hydrogen bond with a nonbridging oxygen in the β-phosphate (Figure 1D). Overall, each network of enzyme−substrate interactions is important for effectively discriminating between rNTPs and dNTPs. Furthermore, these structural findings suggest efficient RNA synthesis is a functionality conferred by multiple residues.

rNTP Incorporation and Extension Are More Complex Than a Single-Residue Model. Although the steric gate residue strongly influences rNTP insertion, it does not necessarily regulate successive rNTP incorporations. The extension of an rNMP-containing primer often leads to premature termination of RNA synthesis after the addition of approximately four to seven rNTPs for various enzymes with a steric gate residue of reduced size (*20*, *23*, *24*, *41*, *42*, *49*). This extension problem likely originates from the suboptimal alignment of the 3′-OH group because of the presence of the 2′-OH group on the ribose ring or the inability of a polymerase's DNA binding cleft to accommodate the A-like helix of an RNA−DNA duplex rather than the standard B-form helix of a DNA−DNA duplex (*50*−*54*). In contrast, Dbh F12A and Dpo4 Y12A are capable of performing at least 10 and 20 rNTP insertions, respectively, without any significant pausing (*28*, *37*), a catalytic function that may be related to their flexible active site or the additional little finger subdomain of Y family DNA polymerases that could assist the binding of an A-form helix.

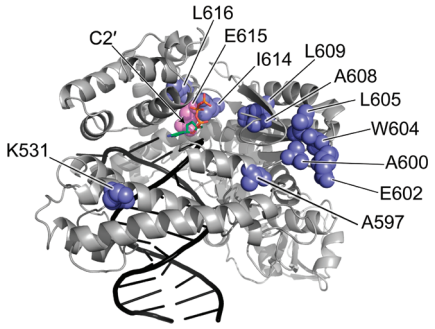

FIGURE 4: Mutations mapped onto the crystal structure of a truncated form of *Taq* (PDB entry 3KTQ). Residues that were mutated (blue space-filling models) are labeled on the ternary complex of *Taq* (gray), DNA (black), and ddNTP (green sticks). Steric gate residue E615 is colored pink, and the C2′ position is identified with an arrow.

Interestingly, some less obvious mutations have also been shown to relax the sugar specificity and/or to stimulate enhanced rNTP synthesis by DNA polymerases and RTs. For example, the extent of ribonucleotide incorporation was greater than that of the WT for the following mutants: 15-fold for *Taq* A661E (*55*), 20−77-fold for *E. coli* Pol I I709F (*56*), 13-fold for KF F762A (*20*), 3-fold for human immunodeficiency virus type 1 (HIV-1) RT A114S (*57*), 13-fold for Vent A488L (*29*), and 60−300000-fold for various *Taq* mutants (Figure 4) that were created using directed evolution methodologies (*21*, *58*, *59*). These selected point mutants discriminate less against rNTPs during incorporation; however, some are not at the extremely low level measured for the steric gate mutants (Figure 2). Nonetheless, the mutations are critical for a DNA polymerase to perform multiple rNTP incorporations.

On the basis of the available ternary crystal structures [PDB entries 3KTQ for *Taq* and 1RTD for HIV-1 RT (*60*, *61*)], side chains of the non-steric gate residues are 4.5−37 Å from the C2′ position and are closer to the base or phosphate groups, rather than the ribose, of the incoming nucleotide (Figure 4). In addition, most of the other mutation sites do not interact directly with the DNA substrate. Together, these biochemical and structural findings suggest that other direct or indirect interactions with the rNTP substrate facilitate incorporation and elongation of an rNMP-terminated primer. Enzyme dynamics may play a prominent role in the nucleotide discrimination process, for complementary rNTP-induced conformational changes of KF are different from a correct dNTP (*9*, *62*). Stopped-flow studies on KF indicate that, compared to a correct dNTP, the rate of the conformational change from an open to closed state is reduced by ~100-fold for a complementary incoming rNTP (*9*). Additionally, different conformational transitions occur with mismatched dNTPs (*8*, *9*). Thus, the interrelationship between enzyme dynamics and its substrates remains an enigmatic and relatively unexplored area of research in the DNA polymerase field. Understanding the dynamics and function of other residues, both within and outside of the active site, is important for establishing a comprehensive mechanism of sugar discrimination. Altogether, to create a bona fide RNA polymerase from a DNA polymerase scaffold, more studies need to focus on expanding the substrate repertoire of the polymeric nucleic acid (i.e.,

RNA or DNA) rather than the incoming nucleotide (i.e., rNTP or dNTP).

Beyond Sugar Specificity: Other Conserved Functions of the Steric Gate Residue. Besides acting as a sugar determinant, the steric gate residue has been implicated in various functions, particularly lesion bypass. For each of the Y family DNA polymerases *E. coli* Pol IV (F13V), Dbh (F12A), human Pol κ (Y112A), and *E. coli* Pol V (Y11A), the steric gate residue is sometimes required for, or at least enhances, the bypass of a myriad of DNA lesions based on in vitro and in vivo results (*30*, *63*, *64*). Surprisingly, the steric gate residue Y112 of human Pol κ controls its mutagenic potential by increasing the catalytic efficiency during extension of a mismatched primer terminus by ~400-fold (*30*) and by favoring the misincorporation of an oxidized nucleotide, 7,8-dihydro-8-oxo-2′-deoxyguanosine 5′-triphosphate, opposite template dA (*65*). With regard to polymerase fidelity, steric gate residue E710 of KF, an A family member, is important for excluding purine-pyrimidine mismatches, whereas steric gate residue F12 of Dbh, a Y family member, maintained a relatively low fidelity similar to that of the WT (*44*).

Sugar Recognition at the 2′ Position. To exclude rNTPs, DNA polymerases depend on two critical properties of the 2′ substituent: size and stereochemistry. Various nucleotide analogues, including the anticancer drugs cytarabine (araC) and gemcitabine (GemC), have been useful in dissecting the chemical importance of the 2′ group as well as other positions on the ribose ring during nucleotide incorporation. Nucleotide analogues containing chemical groups smaller than a hydroxyl, such as fluorine for GemC, are incorporated with efficiencies more similar to those of dNTPs, while analogues with larger chemical groups, such as a *O*-methyl or amine, are incorporated with efficiencies similar to or worse than those of rNTPs (*31*−*33*, *66*). In addition, when the 2′-OH group is pointed above the ribose ring such as an arabinonucleotide like araCTP, the different orientation of O2′ evades the collision with the steric gate residue (*26*, *31*−*33*, *67*, *68*). Recently, a truncated form of human Pol λ and gapped DNA was crystallized with araCMP or GemCTP as an incoming nucleotide (*69*). The ribose of araC is bound in a manner similar to that of dC because the 2′-OH group is directed away from the backbone COO group of Y505, but for GemC, the ribose is displaced approximately 1 Å from the standard binding position so that the difluoro group does not interfere with the positioning of active site residues Y505 and N513. Despite the efficient incorporation of araCTP, the extension of this altered analogue is typically more problematic than extending rCMP (*68*, *70*). Therefore, the slow extension of araCMP and GemCMP makes these anticancer drugs effective inhibitors of DNA polymerization by functioning as "masked" chain terminators (*67*, *70*−*74*). In comparison, GemCTP is not a masked chain terminator for human Pol γ and human Pol η, although the incorporation and/or extension step was less efficient than that of undamaged DNA (*74*, *75*).

Internalization of Ribonucleotides into DNA: Bypass and Removal Processes. Ribonucleotides can become embedded into genomic DNA following the successful incorporation of an rNTP opposite damaged or undamaged DNA and the subsequent step of either extension or ligation of an rNMP-terminated primer (*76*). For human Pol β, the efficiency of incorporation of a correct dNTP onto an rNMP-terminated primer is as efficient as that of a dNMP-terminated primer (*68*). Similarly, nicked DNA with a 3′-terminal rNMP can be sealed by various DNA ligases that function in DNA repair pathways (*16*−*18*). In addition, nicked DNA containing araCMP and GemCMP, two anticancer drugs, is a substrate for human DNA ligases III/XRCC1 and I (J. A. Brown and Z. Suo, unpublished data) (*32*). These events lead to the possibility of a natural or modified rNMP persisting in genomic DNA that, because of the altered structure of RNA, may interfere with future replication cycles. For example, a single rG residue within a DNA template impedes DNA replication by yeast Pol ε (*14*); human Pol γ can efficiently incorporate only a single dNTP with an RNA template (*77*). During gap-filling DNA synthesis, human Pol β inserted dCTP opposite rG with a moderate 8-fold reduction in catalytic efficiency while human Pol μ was almost nonfunctional with an RNA template (*16*, *68*). Bypassing a site-specific araCMP or GemCMP embedded in a DNA template is inefficient for Pol α (*70*, *71*, *73*, *74*) and human Pol γ (*75*) but not human Pol η (*74*).

The presence of a DNA−RNA intermediate is physiologically relevant in the form of an Okazaki fragment during lagging strand DNA synthesis; therefore, cellular processes for recognizing and removing the inherently unstable RNA exist. Initiator RNA is removed by the activities of RNase H, flap endonuclease 1 (FEN1), and Pol δ (*78*−*81*). Consequently, these enzymes have been proposed to function in the reversal of incorporation of aberrant rNTP by DNA polymerases (*15*). It has been shown that the concerted nicking activity of RNase H and FEN1 can release a single rNMP from a DNA duplex, thereby generating a gap intermediate that could be processed by the base excision repair pathway (*82*). Alternatively, type 1 topoisomerase possesses endoribonuclease activity to excise a site-specific rNMP in DNA (*83*). Details about how these putative RNA removal pathways function in vivo have not been elucidated. It is possible that other protein cofactors or pathways are also involved.

CONCLUDING REMARKS

Understanding the kinetic and structural mechanisms of sugar recognition is necessary to expand the utility of DNA polymerases in various applications. DNA polymerases are utilized for enzymatic synthesis of DNA in various applications such as polymerase chain reaction (PCR) (*84*, *85*), cDNA cloning, DNA sequencing (*86*), and detection of single-nucleotide polymorphisms (SNPs) (*87*). These methods are essential experimental tools for a basic scientist and are useful diagnostic tools for a clinician that needs genomic sequence information for confirming genetic diseases, treating cancer, or overcoming drug resistance. Consequently, the enzymatic synthesis of short RNA oligonucleotides by a modified DNA polymerase is attractive. (i) The high cost of chemical RNA synthesis is one advantage. (ii) Unlike methods using an RNA polymerase, modified DNA polymerases do not require a promoter sequence to be included in the PCR template. (iii) T7 RNA polymerase cannot efficiently synthesize short RNA oligomers (i.e., < 50). Moreover, because of the intrinsic instability of RNA, synthesis using 2′-modified rNTPs is another future consideration because RNA composed of 2′-fluoro, 2′-*O*-methyl, 2′-*O*-methoxyethyl, and 2′,4′-bicyclic rNMPs displays greater stability, especially against nuclease-catalyzed digestion (*88*, *89*). However, one drawback in using a modified DNA polymerase to synthesize RNA is its inability to initiate synthesis de novo, thereby requiring a presynthesized primer.

The knowledge gained from sugar recognition can also be applied to designing improved nucleoside analogues for anticancer and antiviral usage. Some of the anticancer and antiviral nucleoside analogues have modified ribose structures. The design

of new drugs can be accelerated with an improved understanding of the mechanisms of sugar recognition employed by human DNA polymerases and viral polymerases or RTs. Nuances in their mechanisms of sugar recognition have been exploited in overcoming unwanted drug toxicity and resistance. Of course, the design of nucleoside analogues requires the consideration of other enzymatic pathways, e.g., phosphorylation by human or viral kinases. Nonetheless, the mechanisms of sugar selectivity are highly significant for the development of anticancer and antiviral drugs, especially when specific human DNA repair and lesion bypass polymerases have been proposed to be anticancer drug targets (*90*).

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