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General misincorporation frequency: Re-evaluation of the fidelity of DNA polymerases

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ABSTRACT

DNA replication in cells is performed in the presence of four dNTPs and four rNTPs. In this study, we re-evaluated the fidelity of DNA polymerases using the general misincorporation frequency consisting of three incorrect dNTPs and four rNTPs but not using the traditional special misincorporation frequency with only the three incorrect dNTPs. We analyzed both the general and special misincorporation frequencies of nucleotide incorporation opposite dG, rG, or 8-oxoG by *Pseudomonas aeruginosa* phage 1 (PaP1) DNA polymerase Gp90 or *Sulfolobus solfataricus* DNA polymerase Dpo4. Both misincorporation frequencies of other DNA polymerases published were also summarized and analyzed. The general misincorporation frequency is obviously higher than the special misincorporation frequency for many DNA polymerases, indicating the real fidelity of a DNA polymerase should be evaluated using the general misincorporation frequency.

1. Introduction

Faithful replication of genome and accurate copying of DNA are essential to propagate cells. DNA polymerases select the correct dNTP complementary to the templating base with high fidelity. In mechanism, DNA polymerase binds to DNA to form a binary complex and selects the correct dNTP based on Watson-Crick (W-C) base pair to form a polymerase-DNA-dNTP ternary complex, then induces a fast conformational change to facilitate the formation of phosphodiester bond. After the chemical reaction, pyrophosphate is released and the binary complex is relaxed to initiate a new cycle [1].

High fidelity and proofreading activity of DNA polymerases lead to low misincorporation frequency [2]. Generally, A-, B-, and C-family DNA polymerases synthesize DNA with low misincorporation frequency due to only the standard W-C base pairs can fit into their tight active sites [3,4]; whereas the error-prone Y-family DNA polymerases have more open and flexible active sites that can accommodate bulky DNA damage and give higher misincorporation frequencies [5,6].

DNA damage is formed by a multitude of endogenous and exogenous factors that constitute an inevitable challenge for DNA replication machinery [7]. Any structure that differs from the standard nucleotides could be considered as DNA damage. As one of the most common forms of oxidative DNA damage, 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG) generally leads to higher frequency of dATP misincorporation than correct dCTP incorporation [8], thereby leading to G:C to T:A mutagenesis [9].

rNTPs and dNTPs have similar structures except for an extra OH group on the 2′ carbon of sugar. Notably, the concentrations of cellular rNTPs are 1–6 orders of magnitude higher than those of dNTPs, depending on the cell type and cell cycle stage [10–12]. All DNA polymerases from bacteria, yeast, or humans could incorporate rNTPs into DNA to form rNMPs [13–15]. DNA lesion O^6-MeG [16,17], 8-oxoG [8,18], and abasic site [19] change the base structure or completely lose base. rNMPs embedded in DNA also alter the molecular structure of sugar ring, thus rNMPs could also be called as “DNA lesion”. rNMP lesion is more abundant than abasic sites or 8-oxoG in mice [20]. Although certain rNMPs can be removed

Abbreviations: dNTP, deoxynucleoside triphosphate; rNTP, ribonucleoside triphosphate; rNMP, ribonucleoside monophosphate; PaP, *Pseudomonas aeruginosa phage*; Pol, polymerase; Gp90 exo$^-$, exonuclease-deficient gene 90 DNA polymerase; 8-oxoG, 7,8-dihydro-8-oxo-2′-deoxyguanosine.

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through the proofreading function of DNA polymerases or through RNase H2 enzymes [21], removing all rNMPs from DNA backbone is impossible [21]. DNA polymerases may encounter rNMPs in the template strand during the next round of DNA replication. These rNMPs may also change the misincorporation frequency of polymerases.

DNA polymerases should select the solely correct dNTP to Watson-Crick pair with the template base. Incorporation of any of incorrect three dNTPs or four rNTPs can be defined as misincorporation. However, till now, all the fidelities of DNA polymerases that have been published were evaluated based on the misincorporation of only three incorrect dNTPs [2,22]. rNTPs are structurally similar to dNTPs and have large molar excess in cells than dNTPs. DNA polymerases could also misincorporate rNTPs into DNA. Therefore, the fidelity of DNA polymerase should be re-evaluated based on the general misincorporation frequency consisting of all three incorrect dNTPs and four rNTPs, but not based on the traditional special misincorporation frequency with only three incorrect dNTPs. Misincorporation of extra rNTPs by DNA polymerase may change our understanding in the traditional fidelities of DNA polymerases.

In this work, we investigated the general and special misincorporation frequencies of *Pseudomonas aeruginosa* phage 1 (PaP1) A-family DNA polymerase Gp90 [17,18,23] and *Sulfolobus solfataricus* P2 Y-family DNA polymerase Dpo4 [24] by incorporation of dNTPs or rNTPs opposite dG, rG, or 8-oxoG. The general misincorporation frequency was obviously higher than the special misincorporation frequency for some incorporations. Together with the general and special misincorporation frequencies calculated from other published DNA polymerases, the general misincorporation frequency is more suitable to evaluate the real fidelity of DNA polymerase, giving a novel insight in the understanding the real fidelity of DNA polymerase in the presence of rNTPs in cells.

2. Materials and methods

2.1. Materials

T4 polynucleotide kinase, dNTPs, and rNTPs were purchased from New England Biolabs (Beverly, MA). [γ-^{32}P] ATP (special activity 3×10^3 Ci mmol^{-1}) was from PerkinElmer Life Sciences (Boston, MA). Oligodeoxynucleotieds in Table 1 were synthesized and purified by HPLC (Takara Bio, Kyoto, Japan). Dpo4 and Gp90 exo$^-$ were overproduced and purified as described previously [1,17,18,23,25]. Other reagents were of the highest quality commercially available.

Table 1
Oligodeoxynucleotieds used in this study.

24-mer	5′-GCCTCGAGCCAGCCGCAGACGCAT-3′
36-mer	3′-CGGAGCTCGGTCGGCGTCTGCGTAG*CTCCTGCGGCT-5′

G*: dG, rG, 8-oxoG.

Table 2
Steady-state kinetic analysis of nucleotide incorporation opposite dG, rG, or 8-oxoG by Gp90 exo$^-$.

Template	Base	$k_{cat} \times 10^{-5}$ (s^{-1})	K_m (μM)	$k_{cat}/K_m \times 10^{-5}$ ($\mu M^{-1}s^{-1}$)	$f^a_{d(r)NTP}$	Sum of $f^b_{d(r)NTP}$	Total f^c_{NTP}
dG	dCTP	860 ± 30	$(6.8 \pm 0.8) \times 10^{-2}$	13000			
	dGTP	42 ± 1	27 ± 3.4	1.6	1.2×10^{-4}	2.5×10^{-4}	5.8×10^{-4}
	dATP	17 ± 4	41 ± 3.5	0.41	3.2×10^{-5}		
	dTTP	42 ± 2	32 ± 8	1.3	1.0×10^{-4}		
	rCTP	38 ± 8	270 ± 17	0.14	1.1×10^{-5}	3.3×10^{-4}	
	rUTP	70 ± 2	150 ± 19	0.47	3.6×10^{-5}		
	rGTP	11 ± 0.7	4.9 ± 0.9	2.2	1.7×10^{-4}		
	rATP	12 ± 0.9	8.2 ± 1.0	1.5	1.1×10^{-4}		
rG	dCTP	180 ± 2	$(8.2 \pm 0.7) \times 10^{-3}$	22000			
	dGTP	72 ± 4	48 ± 9.6	1.5	6.8×10^{-5}	1.7×10^{-4}	2.3×10^{-4}
	dATP	92 ± 2	390 ± 29	0.24	1.1×10^{-5}		
	dTTP	230 ± 10	110 ± 26	2.1	9.5×10^{-5}		
	rCTP	38 ± 2	45 ± 9.4	0.84	3.8×10^{-5}	0.64×10^{-4}	
	rUTP	15 ± 3	110 ± 11	0.14	6.4×10^{-6}		
	rGTP	5.3 ± 0.1	23 ± 4.2	0.23	1.0×10^{-5}		
	rATP	2.4 ± 0.3	11 ± 2.2	0.22	1.0×10^{-5}		
8-oxoG	dCTP	72 ± 5	$(230 \pm 59) \times 10^{-3}$	310			
	dGTP	23 ± 0.7	260 ± 29	0.09	2.9×10^{-4}	9.2×10^{-3}	9.2×10^{-3}
	dATP	240 ± 5	95 ± 7.9	2.5	8.1×10^{-3}		
	dTTP	15 ± 1	62 ± 22	0.24	7.7×10^{-4}		
	rCTP			ND			
	rUTP			ND			
	rGTP			ND			
	rATP			ND			

The conversion was controlled < 20% by adjustment of enzyme concentration and reaction time.
[a] $f_{d(r)NTP}$: misincorporation frequency.
[b] Sum of f_{dNTP}: the special misincorporation frequency, the sum of misincorporation frequencies of three incorrect dNTPs.
[b] Sum of f_{rNTP}: the sum of misincorporation frequencies of four rNTPs.
[c] Total f_{NTP}: the general misincorporation frequency, the sum of f_{dNTP} and f_{rNTP}.
ND: No incorporation was observed under extreme strong condition.

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