

# Structural Basis for Error-Free Bypass of the 5-*N*-Methylformamidopyrimidine-dG Lesion by Human DNA Polymerase $\eta$ and *Sulfolobus solfataricus* P2 Polymerase IV

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## Supporting Information

**ABSTRACT:** *N*<sup>6</sup>-(2-Deoxy-*D*-erythro-pentofuranosyl)-2,6-diamino-3,4-dihydro-4-oxo-5-*N*-methylformamidopyrimidine (MeFapy-dG) arises from *N*7-methylation of deoxyguanosine followed by imidazole ring opening. The lesion has been reported to persist in animal tissues. Previous in vitro replication bypass investigations of the MeFapy-dG adduct revealed predominant insertion of C opposite the lesion, dependent on the identity of the DNA polymerase (Pol) and the local sequence context. Here we report crystal structures of ternary Pol-DNA-dNTP complexes between MeFapy-dG-adducted DNA template:primer duplexes and the Y-family polymerases human Pol  $\eta$  and P2 Pol IV (Dpo4) from *Sulfolobus solfataricus*. The structures of the hPol  $\eta$  and Dpo4 complexes at the insertion and extension stages, respectively, are representative of error-free replication, with MeFapy-dG in the anti conformation and forming Watson-Crick pairs with dCTP or dC.

Alkylating agents are the earliest class of chemotherapy drugs and are still commonly used to treat different types of cancers. These include monofunctional methylating agents such as temozolomide and bifunctional alkylating agents such as nitrogen mustards or chloroethylating agents.<sup>1</sup>

The guanine *N*7 position constitutes the most nucleophilic site in DNA.<sup>2</sup> Thus, DNA methylation occurs predominantly at that site, resulting in a cationic *N*7-methyldeoxyguanosine adduct.<sup>3</sup> This product can undergo further hydrolysis, yielding an abasic (AP) site<sup>4,5</sup> or the imidazole-ring-fragmented lesion *N*<sup>6</sup>-(2-deoxy-*D*-erythro-pentofuranosyl)-2,6-diamino-3,4-dihydro-4-oxo-5-*N*-methylformamidopyrimidine (MeFapy-dG) (Figure 1).<sup>3,6</sup> Opening of the purine imidazole ring depends on the *N*7 substituent and the pH of the medium. Whereas the ring opening under physiological conditions is slow, it becomes accelerated at higher temperature and in alkaline solution.

MeFapy-dG has been characterized in vivo in the liver of rats<sup>7,8</sup> and has also been observed in the urine of healthy humans.<sup>9</sup> In general, the MeFapy-dG adduct is considered non-

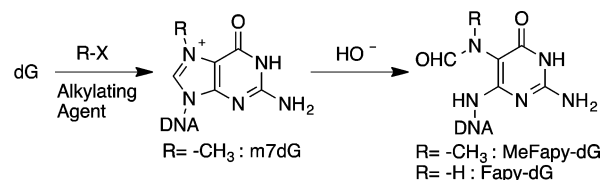


Figure 1. Formation of formamidopyrimidine lesions.

miscoding because the Watson-Crick face remains unaltered. However, various in vitro experiments using *Escherichia coli* DNA polymerase I Klenow fragment and T4 polymerase showed that MeFapy-dG blocks DNA chain elongation.<sup>10,11</sup> Replication of a site-specific MeFapy-dG lesion in primate cells gave complex mutational spectra with frequencies of 7–21% depending on the local sequence. Common mutations were G  $\rightarrow$  T transversions and deletions.<sup>12</sup> An in vitro replication study using the MeFapy-dG lesion with *Sulfolobus solfataricus* Dpo4 found miscoding, with the incorporation of all four nucleotides with various efficiencies depending on the DNA template sequence around the lesion.<sup>13</sup> Oligonucleotides with 5'-T-(MeFapy-dG)-G-3' resulted in error-free bypass, with insertion of dC opposite the adduct and full-length extension of the primer strand. By comparison, a 5'-T-(MeFapy-dG)-T-3' template triggered a one-base deletion, or misincorporation of dA opposite the MeFapy-dG lesion. The error-free bypass and extension efficiency by Dpo4 was estimated to be 74% for 5'-T-(MeFapy-dG)-G-3' and 51% for 5'-T-(MeFapy-dG)-T-3', along with 11% one-base deletion product for the latter template. Recent in vitro replication bypass experiments using human Y-family polymerases (hPols)  $\eta$ ,  $\kappa$ ,  $\iota$ , and Rev1 showed efficient translesion synthesis (TLS) by hPols  $\eta$  and  $\kappa$  with error-free insertion of dCTP opposite MeFapy-dG and extension in the above sequence contexts.<sup>14</sup> Among these TLS Pols, hPol  $\eta$  is the most efficient in the error-free bypass of MeFapy-dG (>70%).

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To analyze the structural basis for the mostly error-free bypass of the MeFapy-dG adduct by hPol  $\eta$ , we determined the crystal structure of an hPol  $\eta$  complex trapped at the insertion stage, with MeFapy-dG opposite the nonhydrolyzable dCMPNPP analogue (in which an N atom bridges the  $\alpha$  and  $\beta$  P atoms), in the presence of  $Mg^{2+}$  (Table 1). Further crystal

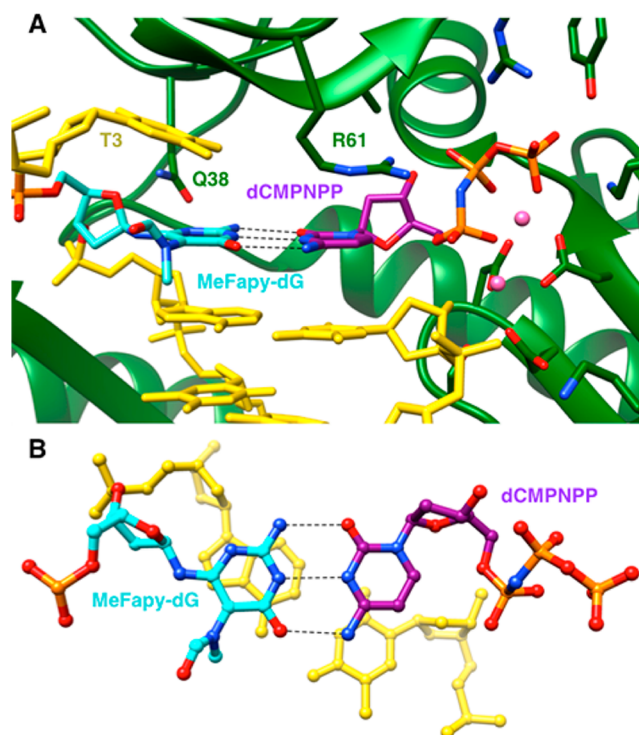
**Table 1.** DNA Sequences Used in the Crystallizations

complex	DNA sequence (X = MeFapy-dG)	incoming nucleotide
hPol $\eta$ (insertion)	3'-TCG CAG TAX TAC-5' 5'-AGC GTC AT-3'	dCMPNPP
ssDpo4 (insertion)	3'-CCC CCT TCC TAA GXT ACT-5' 5'-GGG GGA AGG ATT C-3'	dATP
ssDpo4 (extension)	3'-CCC CCT TCC TAA TXT ACT-5' 5'-GGG GGA AGG ATT AC-3'	dATP

structures concern the hPol  $\kappa$  homologue Dpo4 in complex with an MeFapy-dG-containing template–primer DNA duplex in the presence of  $Ca^{2+}$  and trapped in two different phases of bypass. In the first complex, representative of the insertion stage and a  $-1$  frameshift, MeFapy-dG is unopposed by a residue from the primer, and instead the incoming dATP pairs with the T that is 5'-adjacent to the adduct on the template. In the second complex, representative of the extension stage and error-free bypass, MeFapy-dG pairs with dC at the  $-1$  position, and the incoming dATP pairs with the downstream T of the template.

The structure of the hPol  $\eta$  ternary complex with MeFapy-dG paired opposite incoming dCMPNPP was determined at a resolution of 2.48 Å (Figure 2 and Table 2). The DNA duplex consists of a 12-mer template containing MeFapy-dG and an 8-mer primer (Table 1). In the structure, all of the primer nucleotides were visible in the electron density maps along with 11 of the 12 template nucleotides (Table 2). An example of the quality of the final electron density is shown in Figure S1 in the Supporting Information. At the active site, the MeFapy-dG:dCMPNPP pair displays the expected Watson–Crick geometry, with the formamide moiety adopting an orientation that is more or less perpendicular to the plane of the six-membered ring (Figure 2A). Thus, the active-site configuration in the structure of the hPol  $\eta$ -MeFapy-dG complex is similar to that in the crystal structure of the complex between hPol  $\eta$  and native DNA with a G:dCMPNPP pair lodged at the active site.<sup>15</sup> Superimposition of the active sites of these two structures indicates that amino acids from the hPol  $\eta$  finger domain (i.e., Gln-38 and Arg-61) adopt similar orientations relative to the nascent base pair (Figure S2).

Two structures were determined for Dpo4 in complex with DNA duplexes containing MeFapy-dG-modified template strands (Table 1). Both crystals diffracted to ca. 3 Å and belong to space group  $P2_12_12$  with a single complex per asymmetric unit (Table 2). The first structure features the template sequence 5'-T(MeFapy-dG)G-3' and represents a so-called type-II complex, in which the adduct is unopposed by a primer base, resulting in a  $-1$  frameshift (Figures 3 and S3). As is characteristic for a type-II complex,<sup>16</sup> Dpo4 simultaneously accommodates two template nucleotides inside the catalytic pocket, namely, MeFapy-dG and the 5'-adjacent T. Pairing between this T and the incoming dATP leaves a 6 Å gap between the  $\alpha$ -phosphate of the nucleotide triphosphate and the 3'-hydroxyl group at the primer terminus (Figure 3). The ability of Dpo4 to accommodate two template bases in its active

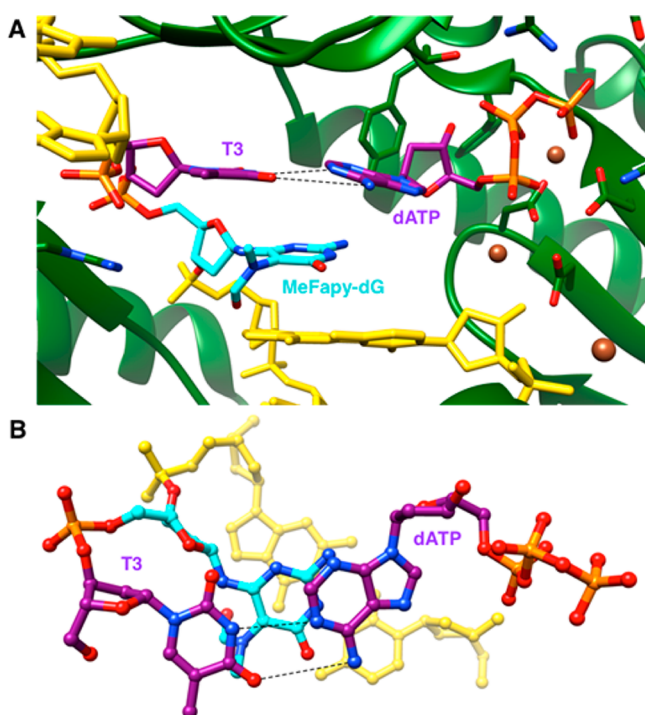


**Figure 2.** Active-site configuration in the ternary hPol  $\eta$  insertion-step complex with dCMPNPP opposite MeFapy-dG. (A) View into the DNA major groove. (B) View rotated by  $\sim 90^\circ$  around the horizontal axis and looking perpendicularly onto the nucleobase plane of the incoming dCMPNPP. Carbon atoms of MeFapy-dG and dCMPNPP are colored in cyan and purple, respectively, and  $Mg^{2+}$  ions are pink spheres. Gln-38 forms a hydrogen bond to O4' of MeFapy-dG, but N3 in the minor groove is too far removed from the Gln-38 amide oxygen (3.9 Å) for hydrogen-bond formation.

**Table 2.** Selected Crystal Data, Diffraction Data Collection, and Refinement Parameter Statistics

	hPol $\eta$ (insertion)	ssDpo4 (insertion)	ssDpo4 (extension)
Data Collection			
space group	$P6_1$	$P2_12_12$	$P2_12_12$
resolution [Å] <sup>a</sup>	50.0–2.65 (2.70–2.65)	50.0–3.10 (3.15–3.10)	30.0–2.90 (2.95–2.90)
<i>a</i> [Å]	98.97	95.45	94.38
<i>b</i> [Å]	98.97	102.72	103.97
<i>c</i> [Å]	81.62	53.43	52.56
completeness [%]	98.9 (100)	98.4 (84.7)	99.8 (100)
<i>I</i> / $\sigma$ ( <i>I</i> )	14.5 (1.9)	21.2 (2.9)	18.7 (1.8)
<i>R</i> <sub>merge</sub> [%]	14.7 (98.5)	11.2 (68.0)	14.3 (61.7)
redundancy	5.7 (5.6)	6.2 (3.1)	7.1 (7.1)
Refinement			
<i>R</i> <sub>work</sub> [%]	15.9 (22.1)	16.4 (25.9)	17.1 (26.9)
<i>R</i> <sub>free</sub> [%] <sup>b</sup>	22.9 (34.1)	25.6 (38.6)	23.5 (35.3)
avg <i>B</i> [Å <sup>2</sup> ]	47.7	88.2	69.8
RMSD bonds [Å]	0.011	0.010	0.010
RMSD angles [deg]	1.5	1.5	1.4
PDB ID <sup>c</sup>	4RU9	4RUA	4RUC

<sup>a</sup>Statistics for the highest-resolution shell are shown in parentheses. <sup>b</sup>Based on 5% of the reflections. <sup>c</sup>Atomic coordinates and structure factors have been deposited in the Protein Data Bank (<http://wwpdb.org/>).

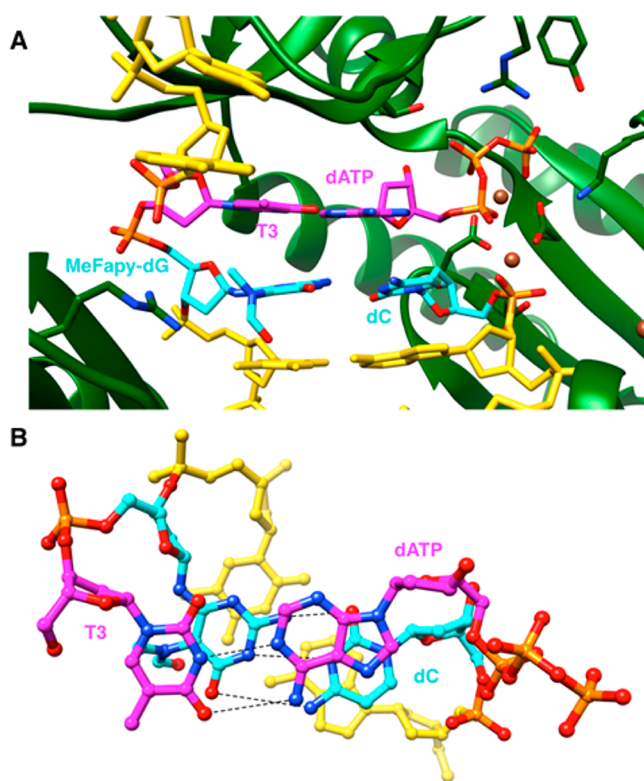


**Figure 3.** Active-site configuration in the ternary Dpo4 insertion-step complex with unpaired MeFapy-dG and incoming dATP opposite template T. (A) View into the DNA major groove. (B) View rotated by  $\sim 90^\circ$  around the horizontal axis and looking perpendicularly onto the nucleobase plane of the incoming dATP. Color codes match those in Figure 2 except that carbon atoms of the template T 5'-adjacent to the adduct are purple.  $\text{Ca}^{2+}$  ions are brown spheres.

site forms the basis for correct bypass of cyclic pyrimidine dimers (CPDs) by this Pol, with the type-II configuration and a misaligned template strand being consequences of the spacious active site.<sup>16</sup>

The second structure is of a postinsertion ternary complex involving the template sequence 5'-T(MeFapy-dG)T-3' and a 14-mer primer of which the 3'-terminal cytosine is intended to pair with the template MeFapy-dG (Figures 4 and S4). The incoming dATP pairs with the thymine base to the 5'-side of MeFapy-dG. Both the MeFapy-G:dC and dT:dATP base pairs are found in the standard Watson-Crick hydrogen-bonding configuration. As in the case of the hPol  $\eta$  complex (Figure 2), the formamide is rotated out of the plane of the six-membered ring in the two Dpo4 complexes (Figures 3 and 4). However, unlike in the hPol  $\eta$  complex, the formamide C=O is directed toward the residue 3'-adjacent to the adduct.

In vitro or in vivo formations of methylated or non-methylated formamidopyrimidines (MeFapy-dG or Fapy-dG, respectively) in DNA have been studied extensively.<sup>17</sup> Although the origins of these lesions are different (Fapy-dG is the result of oxidative damage), they are structurally very similar and only weakly mutagenic. Earlier studies established that Pols exhibit a strong preference for dCTP incorporation opposite the Fapy-dG lesion.<sup>18–21</sup> Recent work by Gehrke et al.<sup>22</sup> showed that the replication of the carbocyclic sugar analogue Fapy lesion (cFaPydG) by high-fidelity polymerase I from *Geobacillus stearothermophilus* (*Bst* Pol I) results in error-free bypass alongside a smaller amount of error-prone bypass. A previous NMR structure of a cFaPydG:dC-containing DNA duplex was



**Figure 4.** Active-site configuration in the ternary Dpo4 extension-step complex with primer dC opposite MeFapy-dG and dATP opposite template dT. (A) View into the DNA major groove. (B) View rotated by  $\sim 90^\circ$  around the horizontal axis and looking perpendicularly onto the nucleobase plane of the incoming dATP. Carbon atoms of the MeFapy-G:dC and dT:dATP pairs are colored in cyan and magenta, respectively.

also consistent with cFaPydG pairing with dC in a standard Watson-Crick fashion.<sup>23</sup>

N7-methyldeoxyguanosine (m7dG), the initial lesion formed by methylating agents (Figure 1), has also been the subject of several studies and classified as non-promutagenic.<sup>3,24</sup> Previous crystallographic studies using the chemically stable 2'-fluoro-m7dG analogue in a DNA sequence, in complex with either *E. coli* DNA glycosylase AlkA<sup>25</sup> or human DNA polymerase  $\beta$ ,<sup>26</sup> revealed that this polymerase bypasses m7dG accurately and that the lesion forms a canonical Watson-Crick base pair with incoming dCTP.

In summary, the three X-ray crystal structures of TLS Pols trapped either at the insertion or extension stages of MeFapy-dG bypass synthesis provide detailed insight into the basis of the mostly error-free replication of the adduct by hPol  $\eta$  and Dpo4, an hPol  $\kappa$  homologue. The structure of the hPol  $\eta$ -MeFapy-dG-dCMPNPP ternary complex is consistent with kinetic data of error-free bypass and extension by hPol  $\eta$ . The major groove is wide open at the active site of hPol  $\eta$ , allowing this Pol to potentially also bypass more bulky lesions such as BenzylFapy-dG (Figure S5).

In the hPol  $\eta$  complex, the formamide C=O points toward the 5'-adjacent T, whereas it is directed toward the 3'-adjacent G in the Dpo4 structure(s), perhaps as a result of the proximity of the formamide and 5'-template T in the latter case (Figures 2, 4, and S6). Thus, partial unstacking of the template T 5'-adjacent to the lesion at the active site of hPol  $\eta$  creates somewhat more room, allowing a virtually upright orientation



of the formamide. By comparison, the equivalent T and the six-membered ring of MeFapy-dG are tightly stacked at the Dpo4 active site, and a formamide orientation similar to that in the hPol  $\eta$  complex would create a tight spacing between C=O and the T C5-methyl substituent (Figure S6).

## ■ ASSOCIATED CONTENT

### ■ Supporting Information

Crystallographic experimental procedures, crystal data, electron density maps, and structural superimpositions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b02701.

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### Notes

The authors declare no competing financial interest.

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