Mismatch Extension Ability of Yeast and Human DNA Polymerase η*

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DNA polymerase η (Polη) functions in error-free replication of UV-damaged DNA, and in vitro it efficiently bypasses a cis-syn T-T dimer by incorporating two adenes opposite the lesion. Steady state kinetic studies have shown that both yeast and human Polη are low-fidelity enzymes, and they misincorporate nucleotides with a frequency of $10^{-2}$–$10^{-3}$ on both undamaged and T-T dimer-containing DNA templates. To better understand the role of Polη in error-free translesion DNA synthesis, here we examine the ability of Polη to extend from base mismatches. We find that both yeast and human Polη extend from mismatched base pairs with a frequency of $10^{-7}$ relative to matched base pairs. In the absence of efficient extension of mismatched primer termini, the ensuing dissociation of Polη from DNA may favor the excision of mismatched nucleotides by a proofreading exonuclease. Thus, we expect DNA synthesis by Polη to be more accurate than that predicted from the fidelity of nucleotide incorporation alone.

DNA polymerase η (Polη)1 functions in error-free replication of UV-damaged DNA, and mutations in the gene encoding this enzyme result in increased UV mutability in both yeast and humans (1). In humans, inactivation of Polη causes the variant form of the cancer prone syndrome xeroderma pigmentosum (2, 3). Polη is unique among eukaryotic DNA polymerases in its ability to efficiently replicate DNA containing a cis-syn T-T dimer, and it does so by incorporating two adenes across from the two thymines of the dimer (3–6).

The high fidelity of replicative DNA polymerases arises, in part, because their active sites are intolerant of the distorted geometry resulting from mismatches between the template residue and the incoming nucleotide (7). Steady state kinetic studies of yeast and human Polη have indicated that it is a low-fidelity enzyme, misincorporating nucleotides with a frequency of $10^{-2}$–$10^{-3}$ on undamaged DNA (5, 8). Remarkably, however, Polη synthesizes DNA opposite a cis-syn T-T dimer, and it does so by incorporating two adenes across from the two thymines of the dimer (3–6).

The low fidelity of Polη may reflect an unusual tolerance of DNA synthesis in vivo, because the mismatched primer terminus could then be subjected to the proofreading 3′→5′ exonuclease activity of other protein factors.

MATERIALS AND METHODS

DNA Substrates—DNA substrates containing all possible correct base pairs or mismatches at the 3′ primer terminus were generated using four different oligodeoxynucleotide primers and four oligodeoxynucleotide templates. The four 45-nucleotide primers have the following sequence: 5′-TTCGT ATNAT GCCTA CACTG GAGTA CCGGA GCATC GTCGT GACTG GGAAA AC, where N is G, A, T, or C. The four 52-nucleotide templates have the following sequence: 5′-TTCTG ATNAT GCCTA CACTG GAGTA CCGGA GCATC GTCGT GACTG GGAAA AC, where N is G, A, T, or C. The various combinations of primers and templates were annealed by mixing 1 mM 32P-end labeled primer with 1.5 mM template in 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl and heating to 90 °C for 2 min before slowly cooling to room temperature over several hours.

Steady state Kinetics Assays—Yeast and human Polη were expressed in and purified from yeast strain BJ5464 as described (4, 5). The steady state kinetics of single nucleotide incorporation were measured by incubating 1 nM yeast or human Polη with 20 nM DNA substrate in 25 mM sodium phosphate, pH 7.0, buffer containing 5 mM magnesium chloride, 5 mM dithiothreitol, 10 μg/ml bovine serum albumin, and 10% glycerol for 10 min at 25 °C. For nucleotide incorporation following a correctly base paired or mispaired primer terminus, the concentration of dGTP was varied from 0 to 5 μM or from 0 to 2000 μM, respectively. Reactions were quenched after 10 min by adding 10 volume of loading buffer (95% formamide, 0.03% bromphenol blue, and 0.3% xanaphol blue). Samples were then run on 10% polyacrylamide sequencing gels to separate the unextended and extended DNA primers. Gel band intensities were quantified using a PhosphorImager and ImageQuant software (Molecular Dynamics). The observed rate of nucleotide incorporation was calculated by dividing the amount of reaction product formed by the 10-min incubation time. The observed rate of nucleotide incorporation was then plotted as a function of nucleotide concentration, and the apparent $K_m$ and $V_{max}$ parameters were obtained from the best fit to the Michaelis-Menten equation using nonlinear regression (Sigma Plot 4.0). The intrinsic efficiency of mismatch extension, $f_{ext}$, which is a constant that represents the efficiency of extending mismatches in competition with matched termini at equal DNA concentra-

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‡ The abbreviation used is: Polη, polymerase η.
MISMATCH EXTENSION BY POL η

RESULTS AND DISCUSSION

We examined the steady state kinetics of nucleotide incorporation by Pol η following the correctly base paired and mismatched termini in primer-template substrates (7, 9, 10). For example, the rate of incorporation of an A residue by yeast Pol η opposite a template T residue following a G-C base pair or A-C, T-C, and C-C mispairs was measured over a broad range of dATP concentrations (Fig. 1A). Gel band intensities were evaluated, and the obtained V_max and K_m parameters are listed in Table I.

TABLE I

Frequencies of extension from matched and mismatched primer-template termini by yeast Pol η on undamaged DNA

Extension was examined in the presence of dATP, the next correct nucleotide for template T.

| Base pairs at the 3' primer terminus (primer-template) | V_{max} (nM/min) | K_m (μM) | V_{max}/K_m | f_{ext} |
|-------------------------------------------------------|------------------|----------|-------------|--------|
| G · G                                                  | 0.50 ± 0.02      | 100 ± 10 | 5.0 × 10^{-3} | 1.7 × 10^{-3} |
| A · G                                                  | 0.65 ± 0.03      | 180 ± 30 | 3.6 × 10^{-3} | 1.2 × 10^{-3} |
| T · G                                                  | 0.38 ± 0.01      | 130 ± 10 | 2.9 × 10^{-3} | 9.6 × 10^{-4} |
| C · G                                                  | 0.65 ± 0.03      | 100 ± 30 | 3.8 × 10^{-3} | 2.9 × 10^{-3} |
| A · A                                                  | 0.68 ± 0.05      | 170 ± 50 | 4.0 × 10^{-3} | 5.3 × 10^{-3} |
| T · A                                                  | 0.22 ± 0.02      | 0.29 ± 0.10 | 0.76 | |
| C · T                                                  | 0.18 ± 0.01      | 48 ± 13  | 3.8 × 10^{-3} | 4.9 × 10^{-3} |
| G · T                                                  | 1.3 ± 0.1        | 150 ± 40 | 8.7 × 10^{-3} | 5.4 × 10^{-3} |
| A · T                                                  | 0.84 ± 0.07      | 0.51 ± 0.14 | 1.6 | |
| T · T                                                  | 0.21 ± 0.01      | 120 ± 20 | 1.8 × 10^{-3} | 1.1 × 10^{-3} |
| C · T                                                  | 0.12 ± 0.01      | 77 ± 20  | 1.6 × 10^{-3} | 1.0 × 10^{-3} |
| G · C                                                  | 0.28 ± 0.01      | 0.20 ± 0.02 | 1.4 | |
| A · C                                                  | 0.25 ± 0.01      | 21 ± 3   | 0.012 | 8.5 × 10^{-3} |
| T · C                                                  | 0.21 ± 0.01      | 100 ± 20 | 2.1 × 10^{-3} | 1.5 × 10^{-3} |
| C · C                                                  | 0.14 ± 0.01      | 87 ± 21  | 1.6 × 10^{-3} | 1.1 × 10^{-3} |

fig. 1. Mismatch extension by yeast Pol η. A, dAMP incorporation by yeast Pol η opposite a template T following a G-C base pair or A-C, T-C, and C-C mispairs. Yeast Pol η (1 nM) was incubated with DNA substrate (20 nM) and varying concentrations of dATP at 25 °C for 10 min. B, observed rate of nucleotide incorporation by yeast Pol η following a G-C base pair or A-C, T-C, and C-C mispairs graphed as a function of dATP concentration. The obtained V_{max} and K_m parameters are listed in Table I.
Mismatch Extension by Pol η

As shown in Table I, for the incorporation of an A residue following a G-C base pair, the apparent $K_m$ for yeast Pol η is 0.20 μM, and the $V_{\text{max}}$ is 0.28 nM/min, whereas for the incorporation of an A following an A-C mispair, the apparent $K_m$ is 21 μM, and the $V_{\text{max}}$ is 0.25 nM/min, respectively. Thus, for the A-C mispair, $f_{\text{ext}}$ is $8.5 \times 10^{-3}$, and similarly, the $f_{\text{ext}}$ values for the T-C and C-C mispairs are $1.5 \times 10^{-3}$ and $1.1 \times 10^{-3}$, respectively. The $f_{\text{ext}}$ values were determined for all the possible mispairs, and overall, yeast Pol η extends from mispairs with an average frequency of $3.1 \times 10^{-3}$ (Table I).

For most DNA polymerases, the frequency of extension from a given mispair ($f_{\text{ext}}$) is approximately the same as the frequency of incorporating that same mispair ($f_{\text{inc}}$; Refs. 7, 10). Fig. 2A compares the $f_{\text{ext}}$ values with the previously reported $f_{\text{inc}}$ values (8) for yeast Pol η for each possible mispair. Points lying above the dashed line represent mispairs with a higher efficiency of extension than insertion, whereas those below the line indicate mispairs with a lower efficiency of extension than insertion. Because most of the points lie near or below the dashed line, yeast Pol η is somewhat less efficient at extending from mispairs than at forming mispairs.

We also examined human Pol η for its ability to extend from base mispairs. As was observed for yeast Pol η, the $f_{\text{ext}}$ values for human Pol η range from $10^{-2}$ to $10^{-3}$, with an average of $2.5 \times 10^{-3}$ (Table II), and a comparison of $f_{\text{ext}}$ values with the previously published $f_{\text{inc}}$ values (5) indicates that human Pol η is also somewhat less efficient at extending from mispairs than at inserting mispaired bases (Fig. 2B). Pol η replicates through a cis-syn T-T dimer with the same efficiency and fidelity as through undamaged T nucleotides (5, 6). Furthermore, our steady state kinetic analyses of base mispair extension across from the T-T dimer indicate that these mispairs are also inefficiently extended and with the same frequency as mispairs in undamaged DNA.2

When compared with other DNA polymerases, the mispair extension ability of Pol η is greater than that of the high-fidelity

![Fig. 2. Comparison of mispair extension and mispair insertion efficiencies by yeast Pol η (A) and human Pol η (B).](image)

**Table II**

Frequencies of extension from matched and mismatched primer-template termini by human Pol η on undamaged DNA

Extension was examined in the presence of dATP, the next correct nucleotide for template T.

| Base pairs at the 3′ primer terminus (primer-template) | $V_{\text{max}}$ | $K_m$ | $V_{\text{max}}/K_m$ | $f_{\text{ext}}$ |
|-------------------------------------------------------|-----------------|------|---------------------|-----------------|
| **nm/min**                                            | μM              |      |                     |                 |
| G · G                                                 | 0.22 ± 0.008    | 47 ± 7 | $4.7 \times 10^{-3}$ | $4.7 \times 10^{-3}$ |
| A · G                                                 | 0.084 ± 0.007   | 100 ± 30 | $8.4 \times 10^{-4}$ | $8.4 \times 10^{-4}$ |
| T · G                                                 | 0.18 ± 0.002    | 15 ± 1 | $1.2 \times 10^{-3}$ | $1.2 \times 10^{-3}$ |
| G · C                                                 | 0.33 ± 0.03     | 83 ± 17 | $5.3 \times 10^{-3}$ | $5.3 \times 10^{-3}$ |
| G · A                                                 | 0.044 ± 0.002   | 60 ± 21 | $1.2 \times 10^{-3}$ | $1.2 \times 10^{-3}$ |
| A · A                                                 | 0.069 ± 0.005   | 120 ± 40 | $2.4 \times 10^{-3}$ | $2.4 \times 10^{-3}$ |
| T · A                                                 | 0.24 ± 0.01     | 46 ± 13 | $5.3 \times 10^{-3}$ | $5.3 \times 10^{-3}$ |
| G · T                                                 | 0.63 ± 0.06     | 120 ± 40 | $5.3 \times 10^{-3}$ | $5.3 \times 10^{-3}$ |
| A · T                                                 | 0.54 ± 0.03     | 86 ± 28 | $7.8 \times 10^{-4}$ | $7.8 \times 10^{-4}$ |
| T · T                                                 | 0.067 ± 0.005   | 65 ± 25 | $4.8 \times 10^{-4}$ | $4.8 \times 10^{-4}$ |
| C · T                                                 | 0.030 ± 0.003   | 55 ± 0.14 | 0.85 | 0.85 |
| G · C                                                 | 0.47 ± 0.04     | 32 ± 4 | $7.8 \times 10^{-3}$ | $7.8 \times 10^{-3}$ |
| A · C                                                 | 0.25 ± 0.007    | 200 ± 40 | $4.0 \times 10^{-4}$ | $4.0 \times 10^{-4}$ |
| T · C                                                 | 0.080 ± 0.005   | 110 ± 30 | $5.6 \times 10^{-4}$ | $5.6 \times 10^{-4}$ |
| C · C                                                 | 0.062 ± 0.004   | 110 ± 30 | $5.6 \times 10^{-4}$ | $5.6 \times 10^{-4}$ |

2 M. T. Washington, R. E. Johnson, S. Prakash, and L. Prakash, unpublished observations.
DNA polymerase α, the $f_{\text{ext}}^\alpha$, of which ranges from $10^{-3}$ to $10^{-6}$ (10). However, its mispair extension ability is considerably lower than that of the most promiscuous extender of mispairs known, yeast Polζ, which extends from mispaired template primer termini with a frequency of $10^{-1}$ to $10^{-2}$ (11). Polζ plays an essential role in mutagenic bypass of DNA lesions, and it specifically functions in damage bypass by extending from nucleotides placed opposite DNA lesions by another DNA polymerase (11).

Polη has low processivity (5, 8), and thus it has a modest probability (0.2–0.3) of dissociating from the DNA template after each nucleotide incorporation. Our observation that both yeast and human Polη extend from mismatched primer termini with a frequency of $\sim 10^{-3}$ relative to a matched primer terminus implies that Polη has a substantially higher probability of dissociating from the primer terminus after the incorporation of an incorrect nucleotide than a correct nucleotide. Dissociation of Polη would prevent mutation fixation, because any mispairs left in DNA would then be subject to removal by the proofreading exonucleolytic activity of Polδ or other proofreading exonucleases. Thus, DNA synthesis by Polη would be more accurate than is indicated from the fidelity of nucleotide incorporation ($f_{\text{inc}}$) values. Because Polη extends from mismatched bases opposite a T-T dimer with the same efficiency as from undamaged DNA, we predict that the error frequency during T-T dimer bypass will also be lower than that suggested from the $f_{\text{inc}}$ values for the incorporation of wrong nucleotides opposite the two T nucleotides of the T-T dimer (5, 6).

We expect the activity of Polη to be restricted to DNA synthesis during damage bypass. The Rad6-Rad18 complex, which is essential for damage bypass and which contains ubiquitin conjugating and DNA binding activities (12), may be crucial for modulating the specific targeting of Polη to sites where replication has stalled at a DNA lesion and for ensuring the dissociation of Polη from DNA once the lesion has been bypassed. Furthermore, association with other protein factors may increase the fidelity of nucleotide incorporation by Polη. Thus, in vivo, damage bypass by Polη would be much more accurate than $10^{-2}$–$10^{-3}$, the frequency of nucleotide misincorporation.

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