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 adenines 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 $10^{-6}$ 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 adenines 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 T-T dimer with the same efficiency and accuracy as opposite undamaged T residues (5, 6). The low fidelity of Polη may reflect an unusual tolerance of the active site for deviant geometry arising from distorting template lesions such as a T-T dimer.

The accuracy of synthesis by DNA polymerases depends on the frequency of incorporation of incorrect nucleotides into DNA and on the frequency of extension of the mismatched primer termini. Extension of mismatched primers is a critical step in mutation fixation, because in the absence of efficient extension, the mismatched nucleotide can be excised by a proofreading exonuclease, or if the mismatch is not excised, cell death may ensue as a result of incomplete DNA synthesis. Thus, for a mutation to be expressed, extension from the misincorporated nucleotide must occur. To better understand how Polη, with a low nucleotide insertion fidelity, can function in an error-free pathway of translesion DNA synthesis in vivo, here we examine the ability of Polη to extend from base mispairs. We find that yeast and human Polη extend from mismatched primer-templates with a frequency of $10^{-3}$ relative to matched primer-templates. This implies that Polη, which has a low processivity, will have a greater likelihood of dissociating from the DNA template after the incorporation of an incorrect nucleotide than a correct one. That would lower the error rate 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 mispairs 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′-GTTTT CCCAG TCACG ACGAT GCTCC GGTAC TCCAG-3′. The four 45-nucleotide templates have the following sequence: 5′-TTCGT ATNAT GCCTA CACTG GAGTA CCGGA GCATG GTGCT GGAAAC-3′ where N is G, A, T, or C. The four 52-nucleotide templates were designed in such a way that any 3′ base pair present in any of the primers was not present in the template. Extension from mismatched blunt-ended primer-template was achieved by mixing 1 μM 32P-end labeled primer with 1.5 μM 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 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, 10 mM dithiothreitol, 100 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 dATP 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 0.2% cynamol blue). Samples were then run on 10% polyacrylamide sequence 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, $f_m$, which is a constant that represents the efficiency of extending mismatched termini in competition with matched termini at equal DNA concentra-

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‡ The abbreviation used is: Polη, polymerase η.
tions, was calculated as described (7, 9, 10) using the following equation:
\[ f_{\text{ext}} = \frac{(V_{\text{max}}/K_m)_{\text{mismatch}}}{(V_{\text{max}}/K_m)_{\text{matched}}} \]

RESULTS AND DISCUSSION

We examined the steady state kinetics of nucleotide incorporation by Pol\(\eta\) following the correctly base paired and mis-paired termini in primer-template substrates (7, 9, 10). For example, the rate of incorporation of an A residue by yeast Pol\(\eta\) opposite a template T residue following a G-C base pair or an A-C, T-C, or C-C mispair was measured over a broad range of dATP concentrations (Fig. 1A). Gel band intensities were eval-
Mismatch Extension by Pol {\eta}

M. T. Washington, R. E. Johnson, S. Prakash, and L. Prakash, unpublished observations.

Table II

Frequencies of extension from matched and mismatched primer-template termini by human Pol {\eta} on undamaged DNA

<table>
<thead>
<tr>
<th>Base pairs at the 3' primer terminus (primer-template)</th>
<th>V_{max} (\mu M/min)</th>
<th>K_m (\mu M)</th>
<th>V_{max}/K_m</th>
<th>f_{ext}</th>
</tr>
</thead>
<tbody>
<tr>
<td>G · G</td>
<td>0.22 ± 0.008</td>
<td>47 ± 7</td>
<td>4.7 × 10^{-3}</td>
<td>4.7 × 10^{-3}</td>
</tr>
<tr>
<td>A · G</td>
<td>0.084 ± 0.007</td>
<td>100 ± 30</td>
<td>8.4 × 10^{-4}</td>
<td>8.4 × 10^{-4}</td>
</tr>
<tr>
<td>T · G</td>
<td>0.18 ± 0.002</td>
<td>15 ± 1</td>
<td>1.2 × 10^{-3}</td>
<td>1.2 × 10^{-3}</td>
</tr>
<tr>
<td>C · G</td>
<td>0.33 ± 0.03</td>
<td>0.32 ± 0.12</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>G · A</td>
<td>0.044 ± 0.002</td>
<td>83 ± 17</td>
<td>5.3 × 10^{-4}</td>
<td>4.4 × 10^{-4}</td>
</tr>
<tr>
<td>A · A</td>
<td>0.069 ± 0.005</td>
<td>60 ± 21</td>
<td>1.2 × 10^{-3}</td>
<td>1.0 × 10^{-3}</td>
</tr>
<tr>
<td>T · A</td>
<td>0.24 ± 0.01</td>
<td>0.20 ± 0.04</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>C · A</td>
<td>0.11 ± 0.006</td>
<td>46 ± 13</td>
<td>2.4 × 10^{-3}</td>
<td>2.0 × 10^{-3}</td>
</tr>
<tr>
<td>G · T</td>
<td>0.63 ± 0.06</td>
<td>120 ± 40</td>
<td>5.3 × 10^{-3}</td>
<td>7.3 × 10^{-3}</td>
</tr>
<tr>
<td>A · T</td>
<td>0.54 ± 0.03</td>
<td>0.74 ± 0.13</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>T · T</td>
<td>0.067 ± 0.005</td>
<td>86 ± 28</td>
<td>7.8 × 10^{-4}</td>
<td>1.1 × 10^{-3}</td>
</tr>
<tr>
<td>C · T</td>
<td>0.030 ± 0.003</td>
<td>63 ± 25</td>
<td>4.8 × 10^{-4}</td>
<td>6.6 × 10^{-4}</td>
</tr>
<tr>
<td>G · C</td>
<td>0.47 ± 0.04</td>
<td>0.55 ± 0.14</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>A · C</td>
<td>0.25 ± 0.007</td>
<td>32 ± 4</td>
<td>7.8 × 10^{-3}</td>
<td>9.2 × 10^{-3}</td>
</tr>
<tr>
<td>T · C</td>
<td>0.080 ± 0.005</td>
<td>200 ± 40</td>
<td>4.0 × 10^{-4}</td>
<td>4.7 × 10^{-4}</td>
</tr>
<tr>
<td>C · C</td>
<td>0.062 ± 0.004</td>
<td>110 ± 30</td>
<td>5.6 × 10^{-4}</td>
<td>6.6 × 10^{-4}</td>
</tr>
</tbody>
</table>
DNA polymerase α, the $f_{\text{ext}}$ 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 Polξ, which extends from mispaired template primer termini with a frequency of $10^{-1}$ to $10^{-2}$ (11). Polξ 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.

REFERENCES
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