Incorporation of ribonucleotides into DNA can severely diminish genome integrity. However, how ribonucleotides instigate DNA damage is poorly understood. In DNA, they can promote replication stress and genomic instability and have been implicated in several diseases. We report here the impact of the ribonucleotide rATP and of its naturally occurring damaged analog 1,N\textsuperscript{6}-ethenoadenosine (1,N\textsuperscript{6}-era) on translesion synthesis (TLS), mediated by human DNA polymerase η (hpol η), and on RNase H2–mediated incision. Mass spectral analysis revealed that 1,N\textsuperscript{6}-era in DNA generates extensive frameshifts during TLS, which can lead to genomic instability. Moreover, steady-state kinetic analysis of the TLS process indicated that deoxypurines (i.e. dATP and dGTP) are inserted predominantly opposite 1,N\textsuperscript{6}-era. We also show that hpol η acts as a reverse transcriptase in the presence of damaged ribonucleotide 1,N\textsuperscript{6}-era but has poor RNA primer extension activities. Steady-state kinetic analysis of reverse transcription and RNA primer extension showed that hpol η favors the addition of dATP and dGTP opposite 1,N\textsuperscript{6}-era. We also found that RNase H2 recognizes 1,N\textsuperscript{6}-era but has limited incision activity across from this lesion, which can lead to the persistence of this detrimental DNA adduct. We conclude that the damaged and unrepaired ribonucleotide 1,N\textsuperscript{6}-era in DNA exhibits mutagenic potential and can also alter the reading frame in an mRNA transcript because 1,N\textsuperscript{6}-era is incompletely incised by RNase H2.

High-fidelity DNA polymerase-mediated replication is a central tenet of biology. It is important to ensure that the genome is copied completely and accurately during replication without any incorrect insertions to avoid further consequences. On the basis of several studies done during the past decade, ribonucleotide incorporation in DNA is considered a major threat to genome stability (1, 2). Mitochondrial (pol γ)\textsuperscript{2} as well as nuclear DNA polymerases (polα, β, and ε) (3, 4) incorporate ribonucleotides into the genome because the cellular concentrations of ribonucleoside triphosphates (rNTPs) are much higher than the dNTPs (5–7). Discrimination between deoxy-ribo- and ribosugars varies among DNA polymerases, mainly due to the active site “steric gate” residue that plays an important role in limiting rNTP insertions (8). Other sources of ribonucleotide incorporation include HIV-1 reverse transcriptase (RT)-mediated reverse transcription and incomplete removal of Okazaki fragment primers (1, 9). Embedded ribonucleotides in the genome present an enigma in the DNA replication process due to the presence of a 2′-OH group, which renders the backbone susceptible to cleavage and various sugar puckered conformations (10–12). Cells repair such ribonucleotides (in DNA) primarily by the ribonucleotide excision repair (REX) process (13–15) and a mechanism that involves RNase H2 enzyme-mediated recognition and incision of ribonucleotides (15–17). The loss of RNase H2 activity is linked to Aicardi–Goutieres syndrome in humans (18). If RNase H2 is unable to remove these ribonucleotides (19), they can have both favorable (20, 21) and lethal biological consequences (22). Recent investigations have shown that ribonucleotides are deleterious lesions that can lead to mutations, large deletions, replication stress, strand breaks (topoisomerase-1–mediated repair), chromosomal rearrangements, loss of hereditary information, and disruption of transcription processes (1, 23–26).

In cells, reactive oxygen species and lipid peroxidation can cause damage of the nucleotide pool (damaged rNTPs/dNTPs) (7, 27) and can be a threat to the synthesis of genetic material (28). Thus, the coding potential of genetic material can be compromised if damaged rNTPs/dNTPs are incorporated into the genome during replication. Oxidation of a deoxyribo- to a ribo-sugar can also occur (29). In the cellular nucleotide pool, free dGTP is more vulnerable to oxidation than the G present in DNA and leads primarily to the formation of 8-oxo-dGTP (30), which can be inserted into the nascent DNA (31–35). Among the rNTPs, the cellular concentration of rATP is very high, and it may therefore be inserted into DNA (33). In this connection, Clausen and co-workers also (36) reported the levels of rATP in mitochondrial DNA. The possibility of insertion of rATP and damaged rATPs into the DNA during replication cannot be excluded (33). The biological consequences of rATP and damaged rATPs in DNA remain elusive. Our own work has shown that low-fidelity human DNA polymerase η (hpol η) incorporates rATP opposite a 6-etheno-adenosine; UPLC, ultraperformance liquid chromatography; CID, collision-induced dissociation; RT, reverse transcriptase; nt, nucleotides.
Translesion synthesis of 1,N⁶-ethenoadenosine

DNA/DNA

5' R 3' 5' 3'

DNA/RNA

5' R 3' 3' 5'

RNA/DNA

5' R 3' 5' 3'

Figure 1. Human pol η–mediated translesion synthesis, reverse transcription, and RNA primer extension activities across adenosine and 1,N⁶-erA.

cyclobutane pyrimidine dimer as well as 8-oxo-dG lesions (37, 38).

DNA and RNA lesions are ubiquitous to the genome. If DNA damage is left unrepaired, cells utilize low-fidelity DNA polymerases to continue replication across damaged sites (to rescue the stalled replication forks) for cell survival, a process termed translesion synthesis (TLS) (39, 40). The TLS process has garnered considerable attention due to the tolerance of damage in DNA in an error-free or error-prone manner (39, 40). TLS also plays an important role in tolerance of ribonucleotides in DNA, and the main key player is hpol η (especially in dealing with lagging strands derived from Okazaki fragments) (16, 24, 38, 41–46). Studies with 8-oxo-rG in DNA indicate that hpol η is required for the tolerance of this adduct (24). The versatile hpol η is now also known for its reverse transcriptase and transcription-associated roles (38, 47–50). Damage in RNA has not been considered to disturb the cellular processes, possibly due to general lack of replication of RNA and its inherently lower stability as well as the more rapid turnover (51–54). However, RNA is more vulnerable to oxidative damage due to its abundance and single-stranded nature (55, 56), and this damage can be detrimental for the cell (57). RNA damage is also associated with the pathogenesis of some neurodegenerative diseases (58–60). RNA is not exempt from damaging events from reactive oxygen species and lipid peroxidation.

2-Chloroethylene oxide, an oxidation product of the known human liver carcinogen vinyl chloride (VC), reacts with nucleophilic sites in DNA and RNA and leads to the formation of etheno (ε) lesions (61). Exposure of animals to VC or vinyl bromide (VB) resulted in chemical modification of RNA, and a common product was 1,N⁶-ethenoadenosine (1,N⁶-εrA) (62). Several studies have shown that 1,N⁶-εrA can be formed in the presence of metabolites of VC or other compounds that generate reactive epoxides (63–65). Bolt and co-workers (66, 67) measured in vivo alkylation of RNA by VC or VB and its effect on protein synthesis; they reported the synthesis of two unusual proteins from mRNA of VC-exposed animals as compared with control rats (67). Exposure of rats to VB resulted in the synthesis of one unusual protein (67). Schulman and Pelka (68) reported that a total of five adenosine structures were altered after reaction of 2-chlorooxirane (a rearrangement product of the VC metabolite 2-chlorooxirane, also known to generate ε adducts) with Escherichia coli fMet tRNA and also suggested that adenosine residues were modified more rapidly than the cytosine in tRNA (69). Biernat et al. (70) also reported 1,N⁶-εrA formation in tRNA after treatment with 2-chlorooxirane. Transcription errors were also observed in the presence of 1,N⁶-εrA (71). ε-Lesions can also be generated indirectly from oxidative stress through lipid peroxidation (72). Leumann and co-workers (73) studied various damaged bases in RNA in the context of translation, but there is only limited knowledge of the biochemistry of damage in stable RNA. It is important to understand the possible consequences of 1,N⁶-εrA in RNA, especially for RT and translation processes.

We now report TLS, RT, and RNA primer extension events and incision of the most abundant nucleotide, rATP, and its naturally occurring damaged analog 1,N⁶-εrA. We utilized hpol η for the TLS, RT, and RNA primer extension studies due to its recently discovered novel functions (37, 38, 44, 47, 49, 74, 75) and RNase H2 for the incision studies. Our laboratory has recently shown that hpol η can act as a reverse transcriptase in human cells (38) and also that it utilizes RNA as a primer in in vitro replication assays, apart from its role in TLS (74). We examined the roles of hpol η as a TLS polymerase and reverse transcriptase and in RNA primer extension events in the presence of a damaged ribonucleotide in DNA as well as in RNA under physiological conditions (Fig. 1).
Translesion synthesis of 1,N⁶-ethenoadenosine

Results

hpol η tolerates the damaged ribonucleotide 1,N⁶-εrA

To gain detailed insights into the biological consequences of 1,N⁶-εrA and rATP, multiple modified oligonucleotides were used for the TLS, RT, and RNA primer extension studies (Table S1). A primary concern was feasible side reactions related to the presence of the ribo-backbone in the DNA and RNA. Recent studies have shown that strand cleavage, as well as transsesterification reactions (isomerization from 3′–5′ to 2′–5′), at ribonucleotide sites are drastically suppressed in nucleosome core particles (76). In contradiction, it is also known that a ribo-backbone in DNA or RNA is temperature–, pH–, and metal ion–sensitive and can lead to transsesterification as well as strand cleavage (77). Accordingly, we excluded the possibility of side reactions during our experiments by maintaining physiological conditions, as well as a desired metal ion concentration (Mg²⁺), low temperature, and pH.

To investigate TLS across a ribonucleotide embedded in DNA, full-length extension and single nucleotide incorporation reactions were carried out using physiological concentrations of dNTPs as well as rNTPs (78, 79) and hpol η (Fig. 2A and 2B; see “Experimental procedures” for details). Full-length extension reactions were carried out using higher concentrations of hpol η, whereas single-nucleotide incorporation reactions were carried out using lower concentrations of hpol η. With an unmodified DNA template (DNA-dA), hpol η rapidly extended the DNA primer using dNTPs as well as rNTPs (Fig. S1A, lanes 1–8). When an rA-embedded DNA template was used (DNA-rA), hpol η fully extended the DNA primer using dNTPs (Fig. 2A, lanes 1–4). The DNA primer was also elongated to the full-length product in the presence of rNTPs, but with less processivity (Fig. 2A, lanes 5–8). These results show that generation of a ribo-ribo pair at an rA site in a DNA template is an unusual behavior of hpol η. Changing the coding base from dA to rA had only a minor effect on rNTP incorporation. TLS activities were examined in the presence of the modified ribonucleotide 1,N⁶-εrA, and hpol η was less efficient in bypassing 1,N⁶-εrA in a DNA template (Fig. 2C, lanes 1–4) as compared with an rA template. However, extension reactions were strongly blocked when rNTPs were used (Fig.

Figure 2. TLS by hpol η. PAGE (20%, 7 M urea) was conducted. A and C, hpol η (200 nM) elongated DNA primer opposite rA-containing (A) and εrA-containing (C) DNA templates in the presence of a mixture of dNTPs or rNTPs (physiological concentrations). All reactions were done at 37 °C for 5, 30, 60, and 180 min (time gradients indicated with wedges). Lanes 1–4, dNTPs; lanes 5–8, rNTPs. B and D, hpol η (50 nM) was incubated with DNA/DNA-rA; DNA/DNA-1,N⁶-εrA with individual dNTPs and rNTPs (physiological concentrations). Lanes 1–3, dTTP; lanes 4–6, dATP; lanes 7–9, dCTP; lanes 10–12, dGTP; lanes 13–15, rUTP; lanes 16–18, rATP; lanes 19–21, rCTP; lanes 22–24, rGTP. All reactions were done at 37 °C for 5, 30, and 60 min. See “Experimental procedures” and Table S1 for the oligonucleotide sequences used. P, FAM-labeled DNA primer.
These results showed that hpol can act as a reverse transcriptase in human cell extracts (38), and here we examined this novel function of hpol using modified RNA as a template, employing physiological concentrations of dNTPs and rNTPs (Fig. 3) under in vitro conditions. With an unmodified RNA template, hpol can generate a DNA primer in the presence of a mixture of dNTPs (Fig. 3A, lanes 1–4), but only one or two rNTPs were added even after 3 h (Fig. 3A, lanes 5–8). In the case of a 1,N6-ε-A–modified RNA template, the DNA primer was elongated with a mixture of dNTPs with low processivity (Fig. 3C, lanes 1–4), whereas extension reactions were strongly blocked in the assays with rNTPs (Fig. 3C, lanes 5–8). These results indicate that hpol acts as a specific reverse transcriptase in the presence of 1,N6-ε-A and prefers dNTPs under physiological conditions.

As a reverse transcriptase, hpol preferentially added dTTP opposite A in DNA-dA and DNA-rA templates (Fig. S1B and Fig. 2B (lanes 1–3)). With the DNA-rA template, the incorporation preference was dTTP > dATP > dGTP > dUTP > rATP > rGTP (Fig. 2B, lanes 1–24). These results showed that hpol can generate an rA-rU pair during TLS by inserting rUTP opposite rA in DNA. In the case of a 1,N6-ε-A–modified DNA template, hpol preferentially added dATP and dGTP, as compared with dTTP and dCTP (Fig. 2D, lanes 1–24). Additionally, no rNTP incorporation was observed opposite 1,N6-ε-A in DNA (with a lower concentration of hpol), indicating that hpol prefers to add deoxyribonucleotides instead of ribonucleotides opposite 1,N6-ε-A.

Our laboratory has previously shown that hpol can act as a reverse transcriptase in human cell extracts (38), and here we examined this novel function of hpol using modified RNA as a template, employing physiological concentrations of dNTPs and rNTPs (Fig. 3) under in vitro conditions. With an unmodified RNA template, hpol elongated a DNA primer in the presence of a mixture of dNTPs (Fig. 3A, lanes 1–4), but only one or two rNTPs were added even after 3 h (Fig. 3A, lanes 5–8). In the case of a 1,N6-ε-A–modified RNA template, the DNA primer was elongated with a mixture of dNTPs with low processivity (Fig. 3C, lanes 1–4), whereas extension reactions were strongly blocked in the assays with rNTPs (Fig. 3C, lanes 5–8). These results indicate that hpol acts as a specific reverse transcriptase in the presence of 1,N6-ε-A and prefers dNTPs under physiological conditions.

As a reverse transcriptase, hpol preferentially added dTTP opposite A in DNA-dA and DNA-rA templates (Fig. S1B and Fig. 2B (lanes 1–3)). With the DNA-rA template, the incorporation preference was dTTP > dATP > dGTP > dUTP > rATP > rGTP (Fig. 2B, lanes 1–24). These results showed that hpol can generate an rA-rU pair during TLS by inserting rUTP opposite rA in DNA. In the case of a 1,N6-ε-A–modified DNA template, hpol preferentially added dATP and dGTP, as compared with dTTP and dCTP (Fig. 2D, lanes 1–24). Additionally, no rNTP incorporation was observed opposite 1,N6-ε-A in DNA (with a lower concentration of hpol), indicating that hpol prefers to add deoxyribonucleotides instead of ribonucleotides opposite 1,N6-ε-A.

Our laboratory has previously shown that hpol can act as a reverse transcriptase in human cell extracts (38), and here we examined this novel function of hpol using modified RNA as a template, employing physiological concentrations of dNTPs and rNTPs (Fig. 3) under in vitro conditions. With an unmodified RNA template, hpol elongated a DNA primer in the presence of a mixture of dNTPs (Fig. 3A, lanes 1–4), but only one or two rNTPs were added even after 3 h (Fig. 3A, lanes 5–8). In the case of a 1,N6-ε-A–modified RNA template, the DNA primer was elongated with a mixture of dNTPs with low processivity (Fig. 3C, lanes 1–4), whereas extension reactions were strongly blocked in the assays with rNTPs (Fig. 3C, lanes 5–8). These results indicate that hpol acts as a specific reverse transcriptase in the presence of 1,N6-ε-A and prefers dNTPs under physiological conditions.

As a reverse transcriptase, hpol preferentially added dTTP opposite A in DNA-dA and DNA-rA templates (Fig. S1B and Fig. 2B (lanes 1–3)). With the DNA-rA template, the incorporation preference was dTTP > dATP > dGTP > dUTP > rATP > rGTP (Fig. 2B, lanes 1–24). These results showed that hpol can generate an rA-rU pair during TLS by inserting rUTP opposite rA in DNA. In the case of a 1,N6-ε-A–modified DNA template, hpol preferentially added dATP and dGTP, as compared with dTTP and dCTP (Fig. 2D, lanes 1–24). Additionally, no rNTP incorporation was observed opposite 1,N6-ε-A in DNA (with a lower concentration of hpol), indicating that hpol prefers to add deoxyribonucleotides instead of ribonucleotides opposite 1,N6-ε-A.
Translesion synthesis of 1,N⁶-ethenoadenosine

Figure 4. RNA primer extension activities of hpol η. PAGE (20%, 7 mol urea) was performed. A and C, hpol η (200 nM) elongated RNA primer opposite rA-containing (A) and erA-containing (C) DNA templates in the presence of a mixture of dNTPs or rNTPs (physiological concentrations). All reactions were done at 37 °C for 5, 30, 60, and 180 min (time gradients indicated with wedges). Lanes 1–4, dNTPs; lanes 5–8, rNTPs. B and D, hpol η (50 nM) was incubated with RNA/DNA-rA; RNA/DNA-1,N⁶-erA with individual dNTPs and rNTPs (physiological concentrations). Lanes 1–3, dTTP; lanes 4–6, dATP; lanes 7–9, dCTP; lanes 10–12, dGTP; lanes 13–15, rUTP; lanes 16–18, rATP; lanes 19–21, rCTP; lanes 22–24, rGTP. All reactions were done at 37 °C for 5, 30, and 60 min. See “Experimental procedures” and Table S1 for the oligonucleotide sequences used. P, FAM-labeled RNA primer.

catalyzes reverse transcription with preference for incorporation of dNTPs over rNTPs.

We previously reported that hpol η can extend an RNA primer (74), and we next examined the ability of hpol η to extend an RNA primer in the presence of an unmodified as well as a modified ribonucleotide in DNA using physiological concentrations of dNTPs and rNTPs. With the unmodified DNA template (DNA-dA), hpol η readily extended the RNA primer using dNTPs, but the process was slow with rNTPs (Fig. S1C, lanes 1–8). Surprisingly, fully extended as well as stalled products were observed for a DNA-rA template (Fig. 4A, lanes 1–8). The RNA primer was partially extended in the presence of a 1,N⁶-erA-modified ribonucleotide. Single-base addition was predominant with the use of dNTPs (Fig. 4C, lanes 1–4), and RNA primer extension was completely retarded opposite 1,N⁶-erA when rNTPs were used (Fig. 4C, lanes 5–8).

For RNA primer extension, hpol η preferentially added dTTP opposite DNA-dA and DNA-rA templates (Fig. S1D and Fig. 4B, lanes 1–3). For the DNA-rA template, the incorporation preference was dTTP/dGTP/dATP > dCTP (Fig. 4B, lanes 1–24). In the case of a 1,N⁶-erA-modified template, hpol η preferentially added dGTP compared with dATP (Fig. 4D, lanes 1–24). No rNTP incorporation was observed opposite 1,N⁶-erA.

M miscoding potential of the damaged ribonucleotide 1,N⁶-erA

To further measure the efficiency and fidelity of hpol η during TLS, RT, and RNA primer extension processes, steady-state kinetic analysis (80, 81) was done (Table 1, Figs. 5 and 6, and Figs. S2–S16; see “Experimental procedures” for details). In the TLS assays, steady-state kinetic analysis showed that incorporation of dTTP opposite 1,N⁶-erA was highly unfavorable. The specificity constant (k_{cat}/K_m) for dCTP insertion (0.001 μM⁻¹ min⁻¹, Table 1) was dramatically reduced, 4200-fold compared with DNA-dA (4.2 μM⁻¹ min⁻¹; Table 1) and 1600-fold compared with DNA-rA templates (1.7 μM⁻¹ min⁻¹, Table
1, N<sup>6</sup>-ethenoadenosine is a highly error-prone manner, with preference for insertion of deoxypurines, which can affect the reading frame of mRNA. This observation is in line with the previous bypass studies with the 1,N<sup>6</sup>-edA adduct (82).

We next focused on the efficiency of hpol η as a reverse transcriptase (Table 1, Fig. 6, and Figs. S8–S11). Steady-state kinetic analysis revealed that the incorporation of dTTP across 1,N<sup>6</sup>-erA was quite unfavorable, as seen in the TLS process. The specificity constant for insertion of dTTP (0.002 μM<sup>-1</sup> min<sup>-1</sup>; Table 1 and Fig. S8) decreased 2100-fold compared with the RNA-rA template (4.3 μM<sup>-1</sup> min<sup>-1</sup>; Table 1), driven by the much larger K<sub>m</sub> value for 1,N<sup>6</sup>-erA template (Table 1) as compared with the RNA-rA template. For insertion of dATP opposite 1,N<sup>6</sup>-erA, the specificity constant was 10-fold higher than with the RNA-rA template (Table 1 and Fig. 6), again driven by the K<sub>m</sub> difference (Table 1). Thus, hpol η prefers to insert dATP opposite 1,N<sup>6</sup>-erA in an RNA template, similar to HIV-1 RT (83). In the case of dGTP insertion across 1,N<sup>6</sup>-erA, the specificity constant was 2.5-fold higher compared with the RNA-rA template (Table 1 and Fig. S9). For dCTP insertion, the specificity constant was 1.25-fold lower compared with the RNA-rA template (Table 1 and Fig. S10). rUTP incorporation was only observed opposite the RNA-rA template (Table 1 and Fig. S11).

With 1,N<sup>6</sup>-erA in the RNA template, hpol η incorporated dATP 200-fold, dGTP 25-fold, and dCTP 4-fold more efficiently than dTTP (Table 1), indicating that hpol η catalyzes reverse transcription across the damaged ribonucleotide 1,N<sup>6</sup>-erA by the preferential addition of dNTPs instead of rNTPs. With the use of an RT strategy, the position of 1,N<sup>6</sup>-erA in the RNA could be mapped concerning distinctive mutations in the DNA (84).

The efficiency of hpol η in RNA primer extension was examined (Table 1 and Figs. S12–S16). Steady-state kinetic analysis showed that insertion of dTTP opposite 1,N<sup>6</sup>-erA was highly unfavorable, as observed for both TLS and RT processes (Table 1). The specificity constant (0.0004 μM<sup>-1</sup> min<sup>-1</sup>) was 1100-fold lower compared with the RNA-rA template (Table 1) as compared with the RNA-rA template. The K<sub>m</sub> value for 1,N<sup>6</sup>-erA template (Table 1) was 1200-fold lower than for a DNA-dA template (0.50 μM<sup>-1</sup> min<sup>-1</sup>; Table 1 and Fig. S12). For dATP insertion opposite 1,N<sup>6</sup>-erA, the specificity constant was 10-fold lower compared with a RNA-rA template and 15-fold lower compared with a DNA-rA template (Table 1 and Fig. S13). These results indicate a preference for dATP insertion opposite to the RNA-rA template (1.5-fold higher specificity constant) compared with a DNA-dA template. For dGTP insertion opposite 1,N<sup>6</sup>-erA, the specificity constant was 1.7-fold lower compared with a DNA-dA template and 20-fold lower compared with a DNA-rA template (Table 1 and Fig. S14). These results indicate that dGTP is preferentially added opposite a DNA-rA template compared with DNA-dA, as judged by an 11-fold higher specificity constant. The dCTP incorporation was observed only with a DNA-dA template and DNA-rA templates, not with 1,N<sup>6</sup>-erA. The dCTP was preferentially added opposite the DNA-rA (7.5-fold higher specificity constant; Table 1 and Fig. S15). Among the four rNTPs, the insertion of rUTP was observed for DNA-dA and DNA-rA templates but not for the 1,N<sup>6</sup>-erA template. The specificity constant for rUTP insertion opposite DNA-rA was 1, N<sup>6</sup>-erA.}

### Table 1

| Primer/Template | dNTP/rNTP | Km μM | k<sub>cat</sub> min<sup>-1</sup> | k<sub>cat</sub>/K<sub>m</sub> μM<sup>-1</sup> min<sup>2</sup> |
|-----------------|-----------|-------|-----------------|---------------------------------|
| DNA/dA          | dTTP      | 15 ± 2 | 63 ± 3          | 4.2 ± 0.6                       |
|                 | dATP      | 8 ± 2  | 7.0 ± 0.2       | 0.90 ± 0.20                     |
|                 | dGTP      | 32 ± 5 | 11.0 ± 0.9      | 0.20 ± 0.03                     |
|                 | dCTP      | 185 ± 50 | 10 ± 1          | 0.05 ± 0.001                   |
|                 | rATP      | 187 ± 53 | 1.0 ± 0.1       | 0.0006 ± 0.001                 |
|                 | rUTP      | 410 ± 50 | 0.6 ± 0.1       | 0.0010 ± 0.0001                 |
| DNA/rA          | dTTP      | 39 ± 13 | 65.7 ± 7       | 1.65 ± 0.40                     |
|                 | dATP      | 16 ± 4  | 20 ± 1          | 1.25 ± 0.25                     |
|                 | dGTP      | 23 ± 2  | 7.0 ± 0.1       | 0.32 ± 0.02                     |
|                 | dCTP      | 99 ± 22 | 7.6 ± 0.5       | 0.08 ± 0.01                     |
|                 | rATP      | 117 ± 29 | 0.50 ± 0.02     | 0.004 ± 0.001                   |
|                 | rUTP      | 390 ± 70 | 0.50 ± 0.02     | 0.0000 ± 0.0005                 |

| DNA/eRA         | dTTP      | 12 ± 2  | 3.0 ± 0.1       | 0.20 ± 0.04                     |
|                 | dATP      | 41 ± 10 | 2.0 ± 0.4       | 0.04 ± 0.01                     |
|                 | dGTP      | 10 ± 2  | 47.3 ± 0.1      | 0.0010 ± 0.0002                 |
|                 | dCTP      | 143 ± 23 | 5.0 ± 0.3       | 0.04 ± 0.01                     |
|                 | rATP      | 36 ± 21 | 0.9 ± 0.1       | 0.02 ± 0.01                     |
|                 | rUTP      | 210 ± 70 | 3.7 ± 0.4       | 0.010 ± 0.003                   |
|                 |           | 525 ± 90 | 0.70 ± 0.04     | 0.0000 ± 0.0001                 |

| DNA/eRA         | dTTP      | 709 ± 170 | 1.0 ± 0.2       | 0.0020 ± 0.0002                 |
|                 |           | 77 ± 2   | 3.0 ± 0.1       | 0.40 ± 0.10                     |
|                 | rATP      | 9 ± 4    | 0.50 ± 0.03     | 0.05 ± 0.02                     |
|                 | rUTP      | 119 ± 23 | 1.0 ± 0.1       | 0.008 ± 0.001                   |

| RNA/dA          | dTTP      | 210 ± 42 | 98.5 ± 0.1      | 4.5 ± 0.06                     |
|                 |           | 13 ± 3   | 1.0 ± 0.1       | 0.10 ± 0.03                     |
|                 | rATP      | 4 ± 10   | 3.0 ± 0.1       | 0.07 ± 0.02                     |
|                 | rUTP      | 196 ± 40 | 0.90 ± 0.07     | 0.0006 ± 0.0006                 |
|                 |           | 1500 ± 410 | 0.6 ± 0.1     | 0.0048 ± 0.0001                 |

| RNA/dA          | dTTP      | 159 ± 20 | 85 ± 2          | 0.50 ± 0.05                     |
|                 |           | 14 ± 3   | 2.0 ± 0.1       | 0.15 ± 0.03                     |
|                 | rATP      | 20 ± 6  | 15.1 ± 0.8      | 0.20 ± 0.25                     |
|                 | rUTP      | 33 ± 13 | 1.0 ± 0.1       | 0.03 ± 0.01                     |
|                 |           | 855 ± 200 | 0.15 ± 0.01   | 0.0020 ± 0.0002                 |

| RNA/eRA         | dTTP      | 698 ± 300 | 3.00 ± 0.06     | 0.0040 ± 0.0001                 |
|                 |           | 70 ± 16   | 0.60 ± 0.04     | 0.010 ± 0.002                   |
|                 |           | 30 ± 6    | 1.0 ± 0.1       | 0.04 ± 0.01                     |
The specificity constants for insertion of three dNTPs across 1,N\textsuperscript{6}-\textit{rA} were compared. Interestingly, hpol incorporated dGTP 100-fold and dATP 25-fold more efficiently compared with dTTP (Table 1), suggesting that hpol extends an RNA primer opposite the damaged ribonucleotide 1,N\textsuperscript{6}-\textit{rA}–containing DNA template by the preferential addition of dGTP. hpol generates frameshifts opposite the damaged ribonucleotide 1,N\textsuperscript{6}-\textit{rA}

Steady-state kinetic analysis of hpol–mediated TLS showed a preference for dATP and dGTP insertion opposite 1,N\textsuperscript{6}-\textit{rA}. Other possible miscoding events during the lesion bypass were also considered, and semiquantitative LC-ESI-MS/MS analysis was performed using a previously developed protocol (85, 86) for TLS processing (see “Experimental procedures” and Table S1 for the oligonucleotide sequences used) because both the RT and RNA primer extension processes were slower. A 2′-deoxyuridine (dU)–containing primer and physiological concentrations of dNTPs were used for the full-length extension reactions (see “Experimental procedures” for details). The fully extended products were treated sequentially with uracil-DNA glycosylase (UDG) and piperidine to obtain short pieces of extended products (Fig. S17) (85). Sequences of full-length extended products and their relative yields were determined by LC-ESI-MS/MS analysis and are summarized in Table 2 (see Figs. S18–S35 and Tables S2–S12 for fragmentation patterns).

As expected, hpol yielded error-free products for the DNA/DNA-dA complex, with blunt end additions of G and A, respectively (m/z 938.3 (–3) and 933.0 (–3); Figs. S18–S20 and Tables S2 and S3). hpol is well-known to catalyze the blunt
end addition of A and G to oligonucleotides (86–88). For the DNA/DNA-rA complex, hpol \( \eta \) yielded 89% extended products having T incorporated opposite rA (Table 2) with blunt end additions of G and A (m/z 938.3 (3) and 933.0 (3); see Figs. S21–S23 and Tables S4 and S5 for fragmentation patterns). Apart from the insertion of T, incorporation of G was also observed across rA, accounting for 11% of the products (m/z 946.7 (3); Table 2, Fig. S24, and Table S6).

hpol \( \eta \)–mediated bypass of the DNA/DNA-1,N\textsuperscript{6}-rA complex was analyzed (Figs. S25–S35 and Tables S7–S12), yielding multiple extended products of the same m/z (837.0 (3) and 831.9 (3)) and eluted together (Figs. S25, S27, S30, S31, and S34). Mass spectral analysis revealed that frameshift products (one base deletion) contributed 44% of the total, with blunt end additions of G and A, respectively (Table 2, m/z 837.0 (3) and 831.9 (3); Figs. S27 and S32 and Tables S7 and S10). Extended products with frameshift (one base deletion) followed by mis-insertion of G were also observed (5%), with a blunt end addition (Table 2, m/z 837.0 (3); Fig. S28 and Table S8).

Overall, the frameshift products accounted for 49% of the total products derived using physiological concentrations of dNTPs. Apart from frameshift products, small amounts of products with insertion of G (12%) and A (8%) opposite 1,N\textsuperscript{6}-rA were also observed in the MS/MS analysis (Table 2, m/z 837.0 (3) and 831.9 (3); Figs. S29 and S33 and Tables S9 and S11). Interestingly, we did not detect insertion of T opposite 1,N\textsuperscript{6}-rA, indicating that hpol \( \eta \) is not reading this lesion as a deoxyadenosine residue. Surprisingly, LC-ESI-MS/MS analysis also revealed an unexpected extended product containing a G opposite 1,N\textsuperscript{6}-rA and the subsequent misinsertion of another G (insertion of two Gs; Table 2, m/z 842.0 (3); Fig. S35 and Table S12). We included this product in a different category (Table 2) of base substitutions. Such base substitutions have been observed for 8-oxo-rG in human cells (24). LC-ESI-MS/MS provides insight regarding the frameshift products, and the steady-state kinetic and LC-ESI-MS/MS analyses both showed the insertion of A and G opposite 1,N\textsuperscript{6}-rA during TLS.

Human RNase H2–mediated recognition but partial incision of the damaged ribonucleotide 1,N\textsuperscript{6}-rA

RNase H2–mediated RER plays an important role in removing rNTPs in DNA (89), and we analyzed the endoribonuclease activity of RNase H2 enzyme opposite rA and 1,N\textsuperscript{6}-rA in the DNA template. On the basis of our steady-state kinetic and
Translesion synthesis of 1,N⁶-ethenoadenosine

LC-ESI-MS/MS results, both rA and 1,N⁶-erA in the modified DNA templates were paired with the sequences containing T, A, and G complementary to the modification site (CS_1 to CS_3; see “Experimental procedures” for details; Table S1). We performed the incision assays employing FAM-labeled DNA-dA-, DNA-rA-, and DNA-1,N⁶-erA-modified templates paired with T, A, and G in their complementary sequences (Fig. 7). DNA-dA dsDNAs were used as negative controls, and, as expected, no cleaved product for the DNA-dA strand was observed (Fig. 7, lanes 1–6, 19–24, and 37–42). With DNA-rA, RNase H2 recognized and incised rA very efficiently within 5 min (96% cleaved product, 4 nucleotides) irrespective of its complementary base in dsDNAs (Fig. 7, lanes 7–12, 25–30, and 43–48). The endoribonuclease activity of human RNase H2 was drastically reduced when 1,N⁶-erA–modified dsDNAs were paired with T, A, and G (Fig. 7, lanes 13–18, 31–36, and 49–54). These results indicate that RNase H2 recognizes the modified ribonucleotide 1,N⁶-erA but exhibited only partial incision activity. For 1,N⁶-erA dsDNAs under all pairing conditions, ~30% cleaved product (4 nt) was observed after 1 h. RNase H2 could cleave a DNA strand containing 1,N⁶-erA, whether positioned opposite dT, dG, or dA. To determine the rate of 5′-cleavage by RNase H2, we measured initial burst rates (Fig. S36) employing pre-steady-state kinetic assays using an established protocol (90). The burst amplitude was 38 ± 8 nm for DNA-rA and 1.8 ± 0.3 nm for DNA-1,N⁶-erA. The burst rate demonstrated cleavage at rates of 47 ± 7 s⁻¹ for DNA-rA and 78 ± 24 s⁻¹ for DNA-1,N⁶-erA (Fig. S36, inset), and the steady-state rate was 0.15 ± 0.02 s⁻¹ for DNA-rA and 0.025 ± 0.001 s⁻¹ for DNA-1,N⁶-erA.

*E. coli* RNase HII is reported to be very efficient in removing a wide range of damaged ribonucleotides (91), and we examined the endoribonuclease activity of *E. coli* RNase HII employing the same dsDNAs (Fig. S37). As expected, *E. coli* RNase HII recognized and incised rA very efficiently irrespective of its complementary base in dsDNAs (Fig. 7, lanes 7–12, 25–30, and 43–48), and there was no effect on the DNA-dA strand (Fig. S37, lanes 1–6, 19–24, and 37–42). Endoribonuclease cleavage was ~50% (after 1 h) for 1,N⁶-erA–modified DNA templates paired with T, A, and G (Fig. S37, lanes 13–18, 31–36, and 49–54). Our results indicate that *E. coli* RNase HII (91) also recognized 1,N⁶-erA, but incision was slow.

**Discussion**

The DNA replication process is continuously challenged by various obstacles, such as non-B-form structures of DNA (e.g. G-quartets), fragile sites, transcription factors, and numerous DNA lesions (92). In addition to this, rNTP insertion during DNA replication is a major concern because more than one million rNTPs are incorporated per cell cycle (in human cells) due to their cellular abundance (93). rNTPs and dNTPs are the main precursors of RNAs and DNAs and can be damaged endogenously in the nucleotide pool (e.g. formation 8-oxo-dGTP). Recent studies have shown that 8-oxo-G, an oxidized form of GTP, is incorporated into DNAs and that nucleotide excision repair and RER mechanisms are not able to remove...
this adduct efficiently (24). 1,N⁶-erA, a well-known oxidized adduct derived from rATP, is generated from VC (and its reactive metabolites) as well as from lipid peroxidation. 1,N⁶-erA is known to affect protein translation, as discussed earlier. In the present study, we investigated the impact of rATP and the damaged ribonucleotide 1,N⁶-erA on TLS, reverse transcription, RNA primer extension, and ribonucleotide incision processes catalyzed by hpol η and human RNase H2 employing steady-state kinetics, MS, and incision assays. Interestingly, the TLS process was strongly affected due to the presence of 1,N⁶-erA in DNA as compared with rA (Fig. 2). It has previously been reported that hpol η bypasses the major groove DNA adduct 1,N⁶-edA poorly (82, 94). Steady-state kinetic analysis revealed the error-prone bypass of 1,N⁶-erA with the preferable addition of deoxypyrimines (Table 1). These outcomes are congruent with the results of our previous studies on hpol η–catalyzed TLS past a 1,N⁶-edA DNA lesion (i.e. more efficient insertion of dATP (3.7-fold) and dGTP (2.5-fold) than dTTP opposite 1,N⁶-edA) (82).

Among the dNTPs, the dGTP insertion opposite DNA-dA and DNA-rA templates suggests that hpol η preferably adds dGTP opposite DNA-rA, with a specificity constant 1.6-fold higher than for the DNA-da template (Table 1). In comparing the specificity constants for DNA-da and DNA-rA templates, the insertion of dATP was 1.4-fold more efficient for DNA-rA compared with the DNA-da template. dCTP insertion opposite a DNA-rA template yielded a higher specificity constant (1.6-fold) than for the DNA-da template, with the basis being in the $K_{m}$ values (Table 1).

When we compare 1,N⁶-edA and 1,N⁶-erA lesions, the impact of ribo-backbone on the TLS process can be seen. These studies led to a new direction in understanding whether slow TLS across 1,N⁶-erA is due to the ribo-backbone or the damaged ribonucleotide. Recent investigations with 8-oxo-rG in DNA indicate that the ribonucleotide backbone has a strong influence on biological processes (24, 35).

Further, the specificity constants for insertion of dNTP versus rNTP opposite the DNA-da and DNA-rA templates were compared. Interestingly, the incorporation of dTTP was 700-fold more efficient compared with rUTP for the DNA-da template, and incorporation of dTTP was 410-fold more efficient compared with rUTP for the DNA-rA template (Table 1). The specificity constants for dATP and rUTP insertion opposite the DNA-da and DNA-rA templates indicated that incorporation of dATP was favored 900-fold compared with rATP for the DNA-da template, and incorporation of dATP was 1200-fold more efficient than rATP for the DNA-rA template. It is important to note that the sugar selectivity was well-maintained during the TLS as reflected in the kinetic parameters (Table 1).

LC-ESI-MS/MS analysis showed that the presence of 1,N⁶-erA in DNA produces extensive frameshift (one-base deletion) products (Table 2). On the basis of earlier results, we hypothesize that TLS may occur in two different ways. First, the incoming A or G can be stacked across from 1,N⁶-erA in cross-strand fashion and result in frameshifts (one base deletion), with hpol η skipping the lesion (82, 95). This process could contribute to the misinsertion opposite the next base T, positioned 5′ to the lesion on the template strand. These results are consonant with our previous LC-ESI-MS/MS analysis of a 1,N⁶-edA DNA lesion, which yielded 37% frameshifts (82). Structural studies revealed that incoming dATP and dGTP molecules are present in a staggered configuration across the anti-oriented 1,N⁶-edA lesion (82). Subsequent analyses of these studies for both of the lesions indicates that insertion of A and G was reduced drastically across 1,N⁶-erA as compared with 1,N⁶-edA. We assume that the ribo-backbone of 1,N⁶-erA may play an important role in this case. The addition of two Gs by hpol η, possibly due to the tight binding of hpol η with dGTP during the extension process, leads to the apparent misincorporation of G opposite the T positioned 5′ to the lesion (base substitution) or due to the misalignment of the template strand (95, 96). Overall, we have observed extensive frameshifts (one deletion) followed by misinsertion of G as well as another unusual product that indicates the insertion of two Gs (Table 2). The preference for insertion of purines over pyrimidines is presumably due to better-stacking interactions to achieve a proper orientation of the lesion and incoming nucleotide in the active site of hpol η for further replication. Previous TLS studies with 1,N⁶-edA and an abasic site also showed that hpol η predominantly follows the purine rule (82, 97). The in vitro ribonucleotide incision assays indicate that recognition of unmodified rA and 1,N⁶-erA by human RNase H2 is completely different, apparently due to the presence of an etheno group on the adenosine (Fig. 7). It is important to note that ribonucleotide excision repair involves RNase H2–mediated incision of a 5′-ribonucleotide followed by strand displacement, flap cleavage by the flap endonuclease FEN1, and ligation (13–15). Our results indicate that E. coli RNase HII (91) recognizes and incises 1,N⁶-erA slowly. Overall, RNase H2–mediated incomplete incision of 1,N⁶-erA from DNA poses a threat to genome stability. Alternative repair pathways such as base excision repair may have potential roles in removing this adduct from DNA employing the alkyladenine DNA glycosylase AAG. If this adduct persists in the DNA, the versatile human TLS pol η may tolerate this adduct but in an error-prone way. These studies provide new insight into the consequences of 1,N⁶-erA when TLS is the only means of coping with damaged ribonucleotides in DNA (outlined in Fig. 8).

Damage to DNA has been studied extensively, as compared with RNA. RNA is an innately reactive biomolecule, reacting with both endogenous and exogenous genotoxic agents to yield a plethora of lesions and changes in structural integrity. Leumann and co-workers (98) studied various damaged bases in RNA (8-oxo-rA, 8-oxo-rG, ribo-abasic site, 5-hydroxycytidine, and uridine) in the context of protein translation, as discussed earlier. The e-adducts in RNA were also detected in various tissues of mice after exposure to $[^{14}C]VC$ as discussed earlier (63). In liver, RNA accumulated 1,N⁶-erA, and in vivo levels of e-adducts in RNA were higher than in DNA (63). 1,N⁶-erA was also detected in mouse liver RNA after treatment with ethyl carbamate (64).

To this end, we explored the 1,N⁶-erA modification to understand the RT employing hpol η. As a result, hpol η was able to synthesize DNA utilizing 1,N⁶-erA–modified RNA as a template with the preferred addition of dNTPs (Fig. 3). These studies suggested that hpol η may utilize RNA as a template for double-strand break repair (99, 100). However, hpol η showed...
Translesion synthesis of 1,N\textsuperscript{6}-ethenoadenosine

![Diagram of Translesion synthesis of 1,N\textsuperscript{6}-ethenoadenosine]

Reduced reverse transcriptase activities as compared with HIV-1 RT. The preferable addition of deoxypurinines across 1,N\textsuperscript{6}-erA by hpol \( \eta \) (Table 1) was observed. Previous RT studies showed a similar trend with HIV-1 RT, which preferentially added dATP and dGTP across 1,N\textsuperscript{6}-erA (83). Overall, 1,N\textsuperscript{6}-erA was bypassed by hpol \( \eta \), and yielded full-length DNA molecules in this study may be due to the lack of a more effective polar filter, as proposed by Nair and co-workers (75).

RNA primer extension activities indicate that hpol \( \eta \) may play a role in the extension of RNA primer across the templates containing unmodified ribonucleotides using only dNTPs (Fig. 4). The incorporation pattern showed that the hpol \( \eta \) prefers to add deoxypurinines (preferably dGTP) across 1,N\textsuperscript{6}-erA. This result differs from the TLS and RT processes but the basis is unclear for the G preference over A with the RNA primer.

RNase H2 activity toward an oligonucleotide duplex was strongly attenuated when rA (opposite the DNA strand) was replaced by 1,N\textsuperscript{6}-erA (Fig. S36), as seen in both the steady-state and pre-steady-state kinetics. The steady-state kinetic rate was reduced 6-fold. RNase H2 shows burst kinetics, indicative of a rate-limiting step following cleavage (38, 101). The amplitude of the burst (Fig. S36) was reduced from 38 to 1.8 nm (i.e. 20-fold). Although the calculated pre-steady-state burst rate was still 78 s\(^{-1}\), the error in this measurement is high due to the very low amplitude.

We previously reported that the presence of a cyclobutane dimer (T-T) opposite the DNA strand only reduced the rate of RNase H2 cleavage ~2-fold relative to two Ts (T-T) (38). The effect of the presence of 1,N\textsuperscript{6}-erA was much more dramatic than a cyclobutane dimer. There is precedent for the presence of a DNA adduct to change the burst amplitude of an enzyme, specifically a DNA polymerase (102, 103). One kinetic explanation, which has been supported over the years, is that the presence of an adduct facilitates the equilibrium of the enzyme with nonproductive forms (103, 104). This may well be the case here, although other kinetic possibilities could be involved and cannot be excluded without more extensive experiments. The main point is that the adduct 1,N\textsuperscript{6}-erA is highly disruptive of RNase H2 catalysis, as judged by both steady-state and pre-steady-state kinetics (Fig. S36).

In summary, our findings indicate the unique roles of hpol \( \eta \) in dealing with an oxidatively damaged ribonucleotide. hpol \( \eta \) tolerates the damaged ribonucleotide 1,N\textsuperscript{6}-erA during the TLS process with lower efficiency and also generates extensive frameshifts. RNase H2–mediated partial incision of 1,N\textsuperscript{6}-erA in DNA was supported by the observation of a (low) burst amplitude. hpol \( \eta \) acts as specific reverse transcriptase in the presence of damaged ribonucleotide 1,N\textsuperscript{6}-erA in RNA, showing preference for dNTPs. In addition, poor RNA primer extension activities were observed by hpol \( \eta \).

Experimental procedures

Reagents

The catalytic core of human DNA pol \( \eta \) (amino acids 1 – 432) was expressed in \( E.\ coli \) and purified as reported previously (105, 106). Human RNase H2 was expressed in \( E.\ coli \) and purified as reported previously (107, 108). Unlabeled dNTPs, rNTPs, UDG, and \( E.\ coli \) RNase H1 were purchased from New England Biolabs (Ipswich, MA). C18 Sep-Pak columns were purchased from Waters Corp. (Milford, MA). Micro Biospin-6 columns were purchased from Bio-Rad. Piperidine was from Sigma–Aldrich. Unmodified oligonucleotides and FAM-labeled DNA and RNA primers (twice HPLC-purified) were purchased from Integrated DNA Technologies (Coralville, IA). 1,N\textsuperscript{6}-erA phosphoramidite and FAM-labeled modified oligonucleotides were purchased from ChemGenes Corp. (Wilmington, MA). The tert-butyldimethylsilyl–protected ribo-phosphoramidites were purchased from Glen Research (Sterling, VA).

Oligonucleotide synthesis, purification, and characterization

The oligonucleotide sequences were designed on the basis of our previous in vitro and cell-based studies (38, 74). Solid-phase synthesis of the 1,N\textsuperscript{6}-erA–modified DNAs and RNAs was done on a PerSeptive Biosystems model 8909 DNA synthesizer (see Table S1 for the in-house oligonucleotide sequences). tert-Butyldimethylsilyl–protected ribo-phosphoramidites were used, and the modified ribo- and deoxyribooligonucleotides were synthesed on a 1-\( \mu \)mol scale using the appropriate controlled pore glass as solid support. 5-Ethylthio-1H-tetrazole was used as an activator, and the coupling time for the modified phosphoramidites was 10 min. The deprotection of modified ribo- and deoxyribooligonucleotides was carried out using a reported procedure (83). The crude modified ribo- and deoxyribooligonucleotides were purified by denaturing PAGE (20% PAGE, 7 M urea) at 55 W for 2.5 h using 1X TBE buffer (89 mm Tris-HCl buffer containing boric acid (89 mm) and 2 mm EDTA, pH 8.0). The gel was visualized under a UV lamp at 260 nm, and the desired DNA/RNA bands were isolated from the gel and extracted using TEN buffer (10 mm Tris-HCl buffer containing 1 mm EDTA and 300 mm NaCl, pH 8.0) overnight at room temperature. The modified ribo- and deoxyribooligonucleotides were desalted using C18 Sep-Pak columns, and their
integrity was confirmed by MALDI-TOF in each case using a matrix of 3-hydroxypicolinic acid and ammonium citrate (9:1 molar ratio) (109) and represented as an average mass (positive linear mode; Figs. S38–S39).

Physiological concentrations of dNTPs and rNTPs (78, 79)

All polymerase reactions were carried out using physiological concentrations of dNTPs/rNTPs. Physiological concentrations of dNTPs used in these studies were 25 μM dATP, 30 μM dCTP, 90 μM dGTP, and 40 μM dTTP. Physiological concentrations of rNTPs used in these studies were 2 mM rATP, 0.25 mM rCTP, 0.5 mM rGTP, and 0.5 mM rUTP. The desired stocks of dNTPs/rNTPs were prepared in RNase-free water.

Full-length extension reactions for translesion synthesis, reverse transcription, and RNA primer extension studies (Figs. 2 (A and C), 3 (A and C), and 4 (A and C) and Fig. S1 (A and C)).

A primer-template complex containing a FAM-labeled DNA or RNA primer and an unmodified or modified template were annealed (1:1 molar ratio) at 75 °C for 5 min followed by slow cooling overnight. Full-length extension reactions were carried out using 40 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 5 mM MgCl₂, 5% glycerol (v/v), 10 mM DTT, and 0.1 mg/ml BSA at 37 °C. The final concentration of primer-template complex was 0.5 μM, and 200 nM hpol η was used to obtain fully extended primers. Reactions were initiated by adding a 1-μl mixture of dNTPs/rNTPs (physiological concentrations) to a total volume of 10 μl. Aliquots (1.5 μl) of reaction mixtures were taken at different time points (0, 5, 30, 60, and 180 min) and quenched with 8.5 μl of 10 mM EDTA (pH 8.0) in 95% deionized formamide (v/v). Products were separated using 20% PAGE (7 M urea), and results were visualized using a Typhoon scanner (GE Healthcare) and analyzed by ImageJ software (80).

Single nucleotide incorporation reactions (Figs. 2 (B and D), 3 (B and D), and 4 (B and D) and Fig. S1 (B and D)).

A primer-template DNA complex containing a FAM-labeled DNA or RNA primer and an unmodified or modified template were annealed (1:1 molar ratio) at 75 °C for 5 min, followed by slow cooling overnight. All single-nucleotide insertion reactions were performed using 40 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 5 mM MgCl₂, 5% glycerol (v/v), 10 mM DTT, and 0.1 mg/ml BSA at 37 °C. The final concentration of primer-template complex was 0.5 μM, and 50 nM hpol η was used, followed by the addition of 1 μl of an individual dNTP/rNTP (physiological concentrations) to a total volume of 10 μl. Aliquots (1.5 μl) of reaction mixtures were taken at each time point (0, 5, 30, and 60 min) and quenched with 8.5 μl of 10 mM EDTA (pH 8.0) in 95% deionized formamide (v/v). Products were separated using 20% PAGE (7 M urea), and results were visualized using a Typhoon scanner (GE Healthcare) and analyzed by ImageJ software.

Steady-state kinetics (Table 1, Figs. 5 and 6, and Figs. S2–S16)

A primer-template DNA complex containing a FAM-labeled DNA or RNA primer and an unmodified or modified template were annealed (1:1 molar ratio) at 75 °C for 5 min, followed by slow cooling overnight. All steady-state kinetic reactions were performed using 40 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 5 mM MgCl₂, 5% glycerol (v/v), 10 mM DTT, and 0.1 mg/ml BSA at 37 °C for 5 min. The final concentration of primer-template complex was 0.5 μM, and hpol η was added, ranging from 0.25 to 38 nM to achieve steady-state kinetics of dNTP/rNTP stock insertion. Reactions were initiated by adding 1 μl of an individual dNTP/rNTP stock solution, at each of 10 different concentrations, to a total volume of 5 μl. A general rule of ±20% (P + 1) product formation was maintained (80). Aliquots (1.5 μl) of reaction mixtures were taken at 5-min time points and quenched with 8.5 μl of 10 mM EDTA (pH 8.0) in 95% deionized formamide (v/v). Products were separated using 20% PAGE (7 M urea), and results were visualized using a Typhoon scanner (GE Healthcare) and analyzed by ImageJ software. Data points are shown as means ± S.D. from two independent experiments (see Table 1 for kcat and Km values) and estimated using fit to a hyperbolic equation in Prism software (GraphPad, San Diego, CA) to obtain kcat/Km (ksp) and kcat directly (81).

LC-ESI-MS/MS analysis: Full-length extension followed by UDG and piperidine treatment (Fig. S17)

A 2′-deoxyuridine (dU)-containing FAM-labeled 18-mer primer and an unmodified or modified template were annealed (1:1 molar ratio) at 75 °C for 5 min followed by slow cooling overnight. The full-length extension reactions were carried out under the same reaction conditions as described for the full-length extension assays, except that the final concentrations were as follows: primer-template complex was 2.5 μM; hpol η was 1.2 μM for DNA/DNA-dA and DNA/DNA-rA complexes and 2 μM for DNA/DNA-1,N⁶-erA complex, in a total reaction volume of 85 μl. Reactions were carried out in the presence of mixture of dNTPs (physiological concentrations) at 37 °C for 4 h for DNA/DNA-dA and DNA/DNA-rA complexes and overnight for DNA/DNA-1,N⁶-erA complex. Reactions were terminated using Micro Biospin-6 column separations to extract Mg²⁺ and dNTPs. The resulting products were treated with 25 units of UDG at 37 °C for 4 h, followed by 0.25 mM piperidine at 95 °C for 1 h (85). The reaction mixture was evaporated to dryness by lyophilization. The dried pellet was resuspended in 500 μl of nuclease-free water and taken to dryness by lyophilization. Finally, this dried pellet was resuspended in 25 μl of nuclease-free water for LC-ESI-MS/MS analysis.

LC-ESI-MS/MS analyses were performed on a Finnigan LTQ mass spectrometer (Thermo Scientific Corp., San Jose, CA) connected to an Acquity ultraperformance LC (UPLC) system (Waters). The data were acquired in the ESI negative ion mode using a C18 column (Acquity UPLC BEH, 1.7 μm, 2.1 × 100 mm). UPLC conditions were as follows. Buffer A contained 10 mM NH₄CH₃CO₂, 2% CH₃CN, and 98% H₂O (v/v), pH 7.0, and buffer B contained 10 mM NH₄CH₃CO₂, 95% CH₃CN, 5% H₂O (v/v), pH 7.0. The column temperature was 50 °C. The following gradient program was used with a flow rate of 0.3 ml/min: 0–30% B over 8 min, 30–90% B over 1 min, held at 90% B for 1 min, 90–0% B over 1 min, held at 0% B for 4 min (all v/v). A 15-μl sample aliquot was injected with an autosampler system. ESI settings were as follows: source voltage 4.5 kV, sweep gas
flow rate 5, auxiliary gas flow rate 30, capillary voltage −48 V, sheath gas flow 60, capillary temperature 270 °C, and tube lens voltage −143 V. MS/MS conditions were as follows: activation Q 0.25, activation time 30 ms. Product spectra were acquired over the range m/z 300–2000. The most abundant species were fragmented in the ion trap with a normalized collision energy of 35% (data-dependent mode). The extended product sequences were identified (Figs. S18–S35 and Tables S2–S12) by comparing the observed CID fragments with the theoretical values using the Mongo Oligo Mass Calculator version 2.08 (State University of New York at Albany). The relative yields of extended product sequences were calculated based on the peak areas of extracted ion chromatograms. For the co-eluted products, relative peak areas were calculated on the basis of the intensity of representative fragments (as shown in Fig. S30 for a~C(B(−2) and Fig. S34 for Wf(−1) fragments). The fragmentation patterns of the observed products were confirmed by comparing with the fragmentation patterns of commercial standards (DTT, Coralville, IA).

**RNase H2–mediated incision assays (Fig. 7 and Fig. S37)**

The complementary sequences (CS_1 to CS_3; Table S1) were annealed with FAM-labeled unmodified and modified template (1:1 molar ratio) at 75 °C for 5 min followed by slow cooling overnight (see Table S1 for the oligonucleotide sequences). The final concentration of dsDNA was 0.5 μM. The assays were done at 37 °C in the incision buffer: 40 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT, 5% glycerol (v/v), and 0.1 mg/ml BSA. The reactions were initiated by adding 10 nM RNase H2. Aliquots (1.5 μl) of reaction mixtures were taken at different time points (0, 5, 15, 30, 45, and 60 min) and quenched with 8.5 μl of slow-moving dye (10 mM EDTA (pH 8.0) in 95% deionized formamide (v/v) and xylene cyanol FF, only slow moving dye). Products were separated using 20% PAGE (7 M urea), and the results were visualized using a Typhoon scanner (GE Healthcare) and analyzed by ImageJ software, and the data were fit to a burst equation, \( y = A(1 - e^{-kt}) + k_pE_p \), where A denotes the apparent concentration of the active form of the enzyme, \( k_p \) denotes the burst rate, \( E_p \) represents the steady-state rate, and t is the time. The initial time points (0–0.08 s) were analyzed by non-linear regression of an exponential decay equation fits in Prism software and presented in Fig. S36 (inset).

**Supporting information**

The supporting information contains a list of oligonucleotide sequences used for the studies; PAGE analyses of full-length extension and single-nucleotide incorporation using physiological concentrations of dNTPs and rNTPs; PAGE analyses of steady-state kinetic analysis and the respective Prism graphs; PAGE analyses of full-length extension assays for LC-ESI-MS/MS analysis using physiological concentrations of dNTPs; LC-ESI-MS/MS sequencing analysis of full-length extension reactions for DNA/DNA-dA, DNA/DNA-rA, and DNA/DNA-N₆-erA complexes; respective extracted ion chromatograms and CID fragmentation spectrums; Prism graphs of pre-steady-state kinetic analysis; PAGE of E. coli RNase HII-mediated incision assays; and the MALDI spectrum for DNA-1,N₆-erA and RNA-1,N₆-erA.

**Data availability**

All data are included in the article and supporting information.

**Author contributions**—P. P. G. formal analysis; P. P. G. investigation; P. P. G. methodology; P. P. G. and F. P. G. writing-original draft; P. P. G. and F. P. G. writing-review and editing; F. P. G. conceptualization; F. P. G. resources; F. P. G. supervision; F. P. G. funding acquisition; F. P. G. project administration.

**Acknowledgments**—We thank Prof. Carmelo J. Rizzo for providing access to the DNA synthesizer, Dr. Yan Su for help with expression and purification of hpol η and RNase H2, and Prof. Martin Egli for valuable suggestions. We also thank K. A. Trisler for assistance in the preparation of the manuscript.

**References**

1. Caldecott, K. W. (2014) Ribose—an internal threat to DNA. Science 343, 260–261 CrossRef Medline
2. Forslund, J. M. E., Pfeiffer, A., Stojko5 vic ˇ5 , G., Wanrooij, P. H., and Wanrooij, S. (2018) The presence of rNTPs decreases the speed of mitochondrial DNA replication. PLoS Genet. 14, e1007315 CrossRef Medline
3. Williams, J. S., and Kunkel, T. A. (2014) Ribonucleotides in DNA: origins, repair and consequences. DNA Repair (Amst.) 19, 27–37 CrossRef Medline
4. Wanrooij, P. H., and Chabes, A. (2019) Ribonucleotides in mitochondrial DNA. FEBS Lett. 593, 1554–1565 CrossRef Medline
5. Nick McElhinny, S. A., Watts, B. E., Kumar, D., Watt, D. L., Lundström, E.-B., Burgers, P. M. J., Johansson, E., Chabes, A., and Kunkel, T. A. (2010) Abundant ribonucleotide incorporation into DNA by yeast replicative polymerases. Proc. Natl. Acad. Sci. U.S.A. 107, 4949–4954 CrossRef Medline
25. Conover, H. N., Lujan, S. A., Chapman, M. J., Cornilio, D. A., Shari, R., Williams, J. S., Clark, A. B., Camilo, F., Kunkel, T. A., and Argueso, J. L. (2015) Stimulation of chromosomal rearrangements by ribonucleotides. *Genetics* **201**, 951–961 CrossRef Medline

26. Moss, C. F., Dalla Rosa, I., Hunt, L. E., Yasukawa, T., Young, R., Jones, A. W. E., Reddy, K., Desai, R., Virtue, S., Elgar, G., Voshol, P., Taylor, M. S., Holt, I. J., Reijns, M. A. M., and Spizzinella, A. (2017) Aberrant ribonucleotide incorporation and multiple deletions in mitochondrial DNA of the murine MPV17 disease model. *Nucleic Acids Res.* **45**, 12808–12815 CrossRef Medline

27. Colussi, C., Parlanti, E., Degan, P., Aquilina, G., Barnes, D., Macpherson, P., Karran, P., Crescenzi, M., dogliotti, E., and Bignami, M. (2002) The mammalian mismatch repair pathway removes DNA 8-oxoGMP incorporated from the oxidized dNTP pool. *Curr. Biol.* **12**, 912–918 CrossRef Medline

28. Foti, J. J., Devadoss, B., Winkler, J. A., Collins, J. J., and Walker, G. C. (2012) Oxidation of the guanine nucleotide pool underlies cell death by bactericidal antibiotics. *Science* **336**, 315–319 CrossRef Medline

29. Randerath, K., Reddy, R., Danna, T. F., Watson, P. W., Crane, A. E., and Randerath, E. (1992) Formation of ribonucleotides in DNA modified by oxidative damage in vitro and in vivo: characterization by 1H-NMR-spectroscopy, *Mutat. Res.** **275**, 355–366 CrossRef Medline

30. Markkanen, E. (2017) Nontoxic is not an option: how to deal with oxidative DNA damage. *DNA Repair* **59**, 82–105 CrossRef Medline

31. Shimizu, M., Gruz, P., Kamiya, H., Masutani, C., Xu, Y., Usui, Y., Sugiyama, H., Harashima, H., Hanaoka, F., and Nohmi, T. (2007) Efficient and erroneous incorporation of oxidized DNA precursors in human DNA polymerase η. *Biochemistry* **46**, 5515–5522 CrossRef Medline

32. Pursell, Z. F., McDonald, J. T., Mathews, C. K., and Kunkel, T. A. (2008) Trace amounts of 8-oxo-dGTP in mitochondrial dNTP pools reduce DNA polymerase η-polymerase fidelity. *Nucleic Acids Res.* **36**, 2174–2181 CrossRef Medline

33. Sastre-Moreno, G., Sánchez, A., Esteban, V., and Blanco, L. (2014) ATP insertion opposite 8-oxo-deoxyguanosine by Pol θ mediates error-free tolerance in *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **42**, 9821–9837 CrossRef Medline

34. Katafuchi, A., Sassa, A., Niimi, N., Gruz, P., Fujimoto, H., Masutani, C., Hanaoka, F., Ohta, T., and Nohmi, T. (2010) Critical amino acids in human DNA polymerases η and k involved in erroneous incorporation of oxidized nucleotides. *Nucleic Acids Res.* **38**, 859–867 CrossRef Medline

35. Sassa, A., Yasui, M., and Honma, M. (2019) Current perspectives on mechanisms of ribonucleotide incorporation and processing in mammalian DNA. *Genes Environ.* **41**, 3 CrossRef Medline

36. Berglund, A.-K., Naravarte, C., Engqvist, M. K. M., Hoberg, E., Sizaly, Z., Taylor, R. W., Gustafsson, C. M., Falkenberg, M., and Clausen, A. R. (2017) Nucleotide pools dictate the identity and frequency of ribonucleotide incorporation in mitochondrial DNA. *PLoS Genet.* **13**, e1006628 CrossRef Medline

37. Su, Y., Egli, M., and Guengerich, F. P. (2016) Mechanism of ribonucleotide incorporation by human DNA polymerase η. *J. Biol. Chem.* **291**, 3747–3756 CrossRef Medline

38. Su, Y., Ghodke, P. P., Egli, M., Li, L., Wang, Y., and Guengerich, F. P. (2019) Human DNA polymerase η has reverse transcriptase activity in cellular environments. *J. Biol. Chem.* **294**, 6073–6081 CrossRef Medline

39. Vaisman, A., and Woodgate, R. (2017) Translesion DNA polymerases in eukaryotes: what makes them tick? *Crit. Rev. Biochem. Mol. Biol.* **52**, 274–303 CrossRef Medline

40. Yang, W., and Gao, Y. (2018) Translesion and repair DNA polymerases: diverse structure and mechanism. *Annu. Rev. Biochem.* **87**, 239–261 CrossRef Medline

41. Meroni, A., Nava, G. M., Bianco, E., Grasso, L., Galati, E., Bosio, M. C., Delmastro, D., Muzi-Falconi, M., and Lazzaro, F. (2019) RNase H activities counteract a toxic effect of polymerase η in cells replicating with depleted dNTP pools. *Nucleic Acids Res.* **47**, 4612–4623 CrossRef Medline

42. Sassa, A., Çağlayan, M., Jayan, M., Rodriguez, Y., Beard, W. A., Wilson, S. H., Nohmi, T., Honma, M., and Yasui, M. (2016) Impact of ribonucleotide backbone on translesion synthesis and repair of 7,8-dihydro-8-oxoguanine. *J. Biol. Chem.* **291**, 24314–24323 CrossRef Medline
Translesion synthesis of 1,N\textsuperscript{6}-ethenoadenosine

43. Acharya, N., Manohar, K., Peroumal, D., Khandagale, P., Patel, S. K., Sahu, S. R., and Kumari, P. (2019) Multifaceted activities of DNA polymerase η beyond translesion DNA synthesis. *Curr. Genet.* 65, 649–656

44. Kreisel, K., Engquist, M. K. M., Kalm, J., Thompson, L. I., Boström, M., Navarrete, C., McDonald, J. P., Larsson, E., Woodgate, R., and Clausen, A. R. (2019) DNA polymerase η contributes to genome-wide lagging strand synthesis. *Nucleic Acids Res.* 47, 2425–2435

45. Garcia-Exposito, L., Bouriene, J., Bergoglio, V., Osorio, N., Serbyn, N., Frittmann, O., Stutz, F., and Unk, I. (2012) DNA polymerase-α-T mutant in somatic hypermutation of rearranged immunoglobulin genes is a reverse transcriptase. *Immunol. Cell Biol.* 89, 219–225

46. Meneghetti, E., Crespan, E., Bajak, E., Ravanat, J.-L., Mattsson, Å., Cotgreave, I. A., and Hoffmann, T., Tonnesen, M., Nielsen, P. E., Andersen, H. U., and Unk, I. (2005) Hydrogen peroxide causes greater oxidation in cellular RNA but not DNA in the hippocampus of patients with major mental illness. *J. Psychiatry Neurosci.* 30, 296–302

47. Franklin, A., Milburn, P. J., Blanden, R. V., and Steele, E. J. (2004) Human DNA polymerase-η, an α-T mutator in somatic hypermutation of rearranged immunoglobulin genes, is a reverse transcriptase. *Immunol. Cell Biol.* 82, 219–225

48. Montegut, E., Crespan, E., Bagavathi, L., Kissova, M., Uhanic, F., Sabino, P. M., Imhof, M., Xu, J., Sturla, S. J., Nilforoushan, A., Hübischer, U., van Loon, B., and Maga, M. (2017) Ribonucleotide incorporation by human DNA polymerase η impacts translesion synthesis and RNase H2 activity. *Nucleic Acids Res.* 45, 2600–2614

49. Gali, V. K., Balint, E., Serbyn, N., Frittmann, O., Stutz, F., and Unk, I. (2017) Translesion synthesis DNA polymerase η exhibits a specific RNA extension activity and a transcription-associated function. *Sci. Rep.* 7, 13055

50. Rej, L., Sidorova, J. M., Puget, N., Boudsocq, F., Biard, D. S. F., Monnat, R. J., Cazaubon, C., and Hoffmann, J.-S. (2009) Human DNA polymerase η is required for common fragile site stability during unperturbed DNA replication. *Mol. Cell. Biol.* 29, 3344–3354

51. Li, Z., Wu, J., and DeLeo, C. J. (2006) RNA damage and surveillance under oxidative stress. *IUBMB Life* 58, 581–588

52. Bernstein, J. A., Khodursky, A. B., Lin, P. H., Lin-Chao, S., and Cohen, S. N. (2002) Global analysis of mRNA decay and abundance in *Escherichia coli* at single-gene resolution using two-color fluorescent DNA microarrays. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9697–9702

53. Wurttmann, E. J., and Wolin, S. L. (2009) RNA under attack: cellular handling of RNA damage. *Crit. Rev. Biochem. Mol. Biol.* 44, 34–49

54. Defoiche, I., Zhang, Y., Lagneaux, L., Pettengell, R., Hegedus, A., Willems, L., and Macallan, D. C. (2009) Measurement of ribosomal RNA turnover in vivo by use of deuterium-labeled glucose. *Clin. Chem.* 55, 1824–1833

55. Hofer, T., Badouard, C., Bajak, E., Ravanat, J.-L., Mattsson, Å., Cotgreave, I. A. (2005) Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. *Biomed. Chem. 386*, 333–337

56. Liu, M., Gong, X., Alluri, R. K., Wu, J., Sablo, T., and Li, Z. (2012) Characterization of RNA damage under oxidative stress in *Escherichia coli*. *Bioc. Chem. 393*, 123–132

57. Thapar, R., Bachla, O., Oyenian, C., Brickner, J. R., Chinnam, N. B., Mosamm取parast, N., and Tainer, J. A. (2011) RNA modifications: reversals of cancer and cellular senescence. *Biochemistry* 50, 312–329

58. Nawrot, B., Sochacka, E., and Dürchler, M. (2011) RNA structural and functional changes induced by oxidative stress. *Cell Mol. Life Sci.* 68, 4023–4032

59. Poulson, H. E., Specht, E., Brodaek, K., Henriksen, T., Ellervik, C., Mandrup-Poulsen, T., Tenones, M., Nielsen, P. E., Andersen, H. U., and Weimann, A. (2012) RNA modifications by oxidation: a novel disease mechanism? *Free Radiac. Biol. Med.* 52, 1353–1361

60. Che, Y., Wang, J.-F., Shao, L., and Young, T. (2010) Oxidative damage to RNA but not DNA in the hippocampus of patients with major mental illness. *J. Psychiatry Neurosci.* 35, 296–302.
Translesion synthesis of 1,N6-ethenoadenosine

80. O’Flaherty, D. K., and Guengerich, F. P. (2014) Steady-state kinetic analysis of DNA polymerase single-nucleotide incorporation products. Curr. Protoc. Nucleic Acid Chem. 59, 7.21.21–7.21.27.13 CrossRef Medline

81. Johnson, K. A. (2019) New standards for collecting and fitting steady state kinetic data. Beilstein J. Org. Chem. 15, 16–29 CrossRef Medline

82. Patra, A., Su, Y., Zhang, Q., Johnson, K. M., Guengerich, F. P., and Egli, M. (2016) Structural and kinetic analysis of miscoding opposite the DNA adduct 1,N6-ethenodeoxycytosine by human translesion DNA polymerase η. J. Biol. Chem. 291, 14134–14145 CrossRef Medline

83. Calabretta, A., and Leumann, C. J. (2013) Base pairing and miscoding properties of 1,N6-ethenoadenine- and 3,N4-ethenocytosine-containing RNA oligonucleotides. Biochemistry 52, 1990–1997 CrossRef Medline

84. Alenko, A., Fleming, A. M., and Burrows, C. J. (2017) Reverse transcription past products of guanine oxidation in DNA leads to insertion of A and G opposite 8-oxo-7,8-dihydroguanine and A and G opposite 5-oxo- and 5,6-dihydro-8-oxo-2-deoxyguanosine. Nucleic Acids Res. 45, 5653–5666 CrossRef Medline

85. Chowdhury, G., and Guengerich, F. P. (2011) Liquid chromatography-mass spectrometry analysis of DNA polymerase reaction products. Curr. Protoc. Nucleic Acid Chem. 47, 7.16.11–7.16.16 CrossRef Medline

86. Zhang, H., Goodenough, A. K., Choi, J.-Y., Irimia, A., Loukachevitch, L. V., Kozekov, I. D., Angel, K. C., Rizzo, C. J., Egli, M., and Guengerich, F. P. (2005) DNA adduct bypass polymerization by Sulfolobus solfataricus DNA polymerase Dpo4: analysis and crystal structures of multiple base pair substitution and frameshift products with the adduct 1,N6-ethenoguanine. J. Biol. Chem. 280, 29750–29764 CrossRef Medline

87. Hwang, H., and Taylor, J.-S. (2004) Role of base stacking and sequence context in the inhibition of yeast DNA polymerase η by pyrene nucleotide. Biochemistry 43, 14612–14623 CrossRef Medline

88. Fiala, K. A., Brown, J. A., Ling, H., Kshetry, A. K., Zhang, J., Taylor, J.-S., Su, Y., and Guengerich, F. P. (2016) Pre-steady-state kinetic analysis of human DNA polymerase β and Pol ε processes and frameshifts. Mol. Cell 13, 751–762 CrossRef Medline

89. Zhao, Y., Gregory, M. T., Biertümpfel, C., Hua, Y.-J., Hanaoka, F., and Yang, W. (2013) Mechanism of somatic hypermutation at the WA motif by human DNA polymerase η. Proc. Natl. Acad. Sci. U.S.A. 110, 8146–8151 CrossRef Medline

90. Su, Y., and Guengerich, F. P. (2014) Steady-state kinetic analysis of nucleoside triphosphate incorporation opposite an abasic site by human translesion DNA polymerase η. J. Biol. Chem. 290, 8028–8038 CrossRef Medline

91. Willi, J., Küpfner, P., Évéquoz, D., Fernandez, G., Katz, A., Leumann, C., and Polacek, N. (2018) Oxidative stress damages rRNA inside the ribosome and differentially affects the catalytic center. Nucleic Acids Res. 46, 1945–1957 CrossRef Medline

92. Kim, N., Cho, J.-E., Li, Y. C., and Links-Robertson, S. (2013) RNA/DNA hybrids initiate quasi-palindrome-associated mutations in highly transcribed yeast DNA. PLOS Genet. 9, e1003924 CrossRef Medline

93. Storici, F., Bebenek, K., Kunkel, T. A., Gordenin, D. A., and Resnick, M. A. (2007) RNA-templated DNA repair. Nature 447, 338–341 CrossRef Medline

94. Heider, M. R., Burkhart, B. W., Santangelo, T. J., and Gardner, A. F. (2017) Defining the RNaseH2 enzyme-initiated ribonucleotide excision repair pathway in Archaea. J. Biol. Chem. 292, 8835–8845 CrossRef Medline

95. Su, Y., Patra, A., Harp, J. M., Egli, M., and Guengerich, F. P. (2015) Roles of residues Arg-61 and Gln-38 of human DNA polymerase η in bypass of deoxyguanosine and 7,8-dihydro-8-oxo-2′-deoxyguanosine. J. Biol. Chem. 283, 36711–36723 CrossRef Medline

96. Furge, L. L., and Guengerich, F. P. (1999) Explanation of pre-steady-state kinetics and decreased burst amplitude of HIV-1 reverse transcriptase at sites of modified DNA bases with an additional, nonproductive enzyme-DNA-nucleotide complex. Biochemistry 38, 4818–4825 CrossRef Medline

97. Beckman, J. W., Wang, Q., and Guengerich, F. P. (2008) Kinetic analysis of correct nucleotide insertion by a Y-family DNA polymerase reveals conformational changes both prior to and following phosphodiester bond formation as detected by tryptophan fluorescence. J. Biol. Chem. 283, 36711–36723 CrossRef Medline

98. Biertümpfel, C., Zhao, Y., Kondo, Y., Ramón-Maqués, S., Gregory, M., Lee, J. Y., Masutani, C., Lehmann, A. R., Hanaoka, F., and Yang, W. (2010) Structure and mechanism of human DNA polymerase η. Nature 465, 1044–1048 CrossRef Medline

99. Furge, L. L., and Guengerich, F. P. (1999) Explanation of pre-steady-state kinetics and decreased burst amplitude of HIV-1 reverse transcriptase at sites of modified DNA bases with an additional, nonproductive enzyme-DNA-nucleotide complex. Biochemistry 38, 4818–4825 CrossRef Medline

100. Malfatti, M. C., Henneke, G., Balachander, S., Koh, K. D., Newnam, G., Uehara, R., Crouch, R. I., Storici, F., and Tell, G. (2019) Unlike the Escherichia coli counterpart, archaeal RNase HII cannot process ribose monophosphate abasic sites and oxidized ribonucleotides embedded in DNA. J. Biol. Chem. 294, 13061–13072 CrossRef Medline

101. Bétous, R., Rey, L., Wang, G., Pillaire, M.-J., Puget, N., Selves, J., Biard, D. S. F., Shin-ya, K., Vasquez, K. M., Cazaux, C., and Hoffmann, J.-S. (2009) Role of TLS DNA polymerases η and ε in processing naturally occurring structured DNA in human cells. Mol. Carcinog. 48, 369–378 CrossRef Medline

102. Clausen, A. R., Zhang, S., Burgers, P. M., Lee, M. Y., and Kunkel, T. A. (2013) Ribonucleotide incorporation, proofreading and bypass by human DNA polymerase δ. DNA Repair 12, 121–127 CrossRef Medline

103. Levine, R. I., Miller, H., Grollman, A., Ohashi, E., Ohmori, H., Masutani, C., Hanaoka, F., and Moriya, M. (2001) Translesion DNA synthesis catalyzed by human pol η and pol ε across 1,N6-ethenodeoxycytosine. J. Biol. Chem. 276, 18717–18721 CrossRef Medline

104. Ling, H., Boudsocq, F., Woodgate, R., and Yang, W. (2004) Snapshots of replication through an abasic lesion: structural basis for base substitutions and framingsh. Mol. Cell 13, 751–762 CrossRef Medline