Purification and Characterization of polκ, a DNA Polymerase Encoded by the Human DINB1 Gene*

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The Escherichia coli dinB gene encodes DNA polymerase (pol) IV, a protein involved in increasing spontaneous mutations in vitro. The protein-coding region of DINB1, the human ortholog of DNA pol IV, was fused to glutathione S-transferase and expressed in insect cells. The purified fusion protein was shown to be a template-directed DNA polymerase that we propose to designate polκ. Human polκ lacks detectable 3′ → 5′ proofreading exonuclease activity and is not stimulated by recombinant human proliferating cell nuclear antigen in vitro. Between pH 6.5 and 8.5, human polκ possesses optimal activity at 37 °C over the pH range 6.5–7.5, and is insensitive to inhibition by aphidicolin, dideoxynucleotides, or NaCl up to 50 mM. Either Mg2+ or Mn2+ can satisfy a metal cofactor requirement for polκ activity, with Mg2+ being preferred. Human polκ is unable to bypass a cisplatin adduct in the template. However, polκ shows limited bypass of an 2-acetylaminofluorene lesion and can incorporate dCTP or dTTP across from this lesion, suggesting that the bypass is potentially mutagenic. These results are consistent with a model in which polκ acts as a specialized DNA polymerase whose possible role is to facilitate the replication of templates containing abnormal bases, or possessing structurally aberrant replication forks that inhibit normal DNA synthesis.

We previously reported the cloning and characterization of the human DINB1 and mouse Dinb1 genes, mammalian orthologs of the Escherichia coli dinB gene (1) and members of the UmuC/DinB superfamily of DNA polymerases (2). Expression of the E. coli dinB gene is tightly regulated by the SOS system (3). Following exposure of E. coli cells to DNA-damaging agents such as ultraviolet (UV) radiation, induction of dinB results in enhanced spontaneous (untargeted) mutagenesis of phage λ DNA introduced into the bacteria subsequent to irradiation (4). Increased spontaneous mutagenesis is also observed following overexpression of dinB in cells transfected with F' plasmids, with the most prevalent mutations detected being −1 frameshifts (5). Recombinant E. coli DinB protein carrying a 6-histidine tag was purified and shown to be a DNA polymerase, designated DNA pol IV of E. coli, which is devoid of detectable exonuclease activity (6). Consistent with its apparent ability to generate frameshift mutations in vivo, DNA pol IV is able to extend a misaligned primer-template in vitro, resulting in a −1 frameshift mutation (6). More recently, DNA pol IV has been shown to be unable to efficiently bypass an abasic site, thymine dimer, or 6-4 photoproduct in vitro (7). Based on these observations, it has been suggested that DNA pol IV is a specialized enzyme whose role is to negotiate sites of stalled or arrested DNA replication caused by structurally abnormal replication forks, such as those caused by slippage at repeated sequences (2, 6, 7).

Human DINB1 cDNA is predicted to encode a polypeptide with a molecular mass of 99 kDa, which shares extensive amino acid sequence homology with E. coli DNA pol IV, including proposed catalytic domains (1, 8). As is the case for the E. coli dinB gene (5), overexpression of the mouse Dinb1 cDNA in murine cells is associated with an approximately 10-fold increase in spontaneous mutations (8). These observations suggested that the protein encoded by the human DINB1 gene might also function as a DNA polymerase. In the present study we have used a baculovirus expression system to express and purify a GST1-human DinB1 fusion protein from insect cells and show that the purified protein is a template-directed DNA polymerase in vitro. The enzyme has no detectable 3′ → 5′ exonuclease activity and has optimal DNA polymerase activity at 37 °C and pH 6.5–7.5 (over the range 6.5–8.5) in the presence of Mg21 cations. The enzyme is insensitive to inhibition by aphidicolin or dideoxynucleotides, and retains optimal activity in the presence of NaCl at levels ≤50 mM. We designate this polymerase activity DNA polk. Human polk is unable to bypass a cisplatin adduct in vitro, but has limited ability to bypass an AAF lesion in an error-prone fashion under the same conditions.

EXPERIMENTAL PROCEDURES

Media and Biochemical Reagents—Insect cell TMN-FH media was purchased from Pharmingen. The Klenow fragments of E. coli DNA polymerase I (exo+ and exo−) were obtained from New England Biolabs. Aphidicolin was from Sigma. Dideoxynucleotides were from U. S. Biochemicals. Glutathione-Sepharose was from Amersham Pharmacia Biotech. The protease inhibitor mixture was purchased from Roche Molecular Biochemicals. N-Acetoxy-2-acetylaminoﬂuorene (AAAF) was obtained from the National Cancer Institute. cis-Diaminedichloroplatinum(II) was purchased from Aldrich.

Expression of Wild-type and Mutant GST/polκ—The human DINB1 open reading frame was amplified by high-fidelity polymerase chain reaction using HeLa cell cDNA as template with primers HDInB5′ (5′-GTGGATCCCGCATGGATAGCACAAAGGAGAAGTG-3′) and HDInB3′-His6 (5′-ATGGATCCCGGTCGACTAATGTTGATGATGGTGA-3′)
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GGTGCTTTAAAAATATACCAAGGGTATG-3') that introduce BamHI restriction sites (underlined) on both the 5' and 3' ends of the amplified fragment as well as six histidine residues on the 3' end. The polymerase chain reaction product was cloned into pGEM-T Easy (Promega) to generate pHINB1-6His and sequenced to confirm the integrity of the coding region. The BamHI restriction site-containing DNA fragment of the \( \beta \)-globin \( \beta \)-chain was then cloned into the same site of pAcG2T (Pharmingen) to generate an in-frame fusion with the glutathione S-transferase gene, generating plasmid pAcG2T/HINB1-6His.

The C-terminal deletion mutant was made by high-fidelity polymerase chain reaction with primers HDINB5' and HDINB3'-6His (5'-ATGACTGCAGGTAATGGGATCTCATG-3'), which flank the \( \beta \)-globin restriction site (underlined) and six histidine residues onto the 3' end of the amplified fragment. The polymerase chain reaction product was cloned into pGEM-T Easy (Promega) to give pHINB1C-6His. The D918A/E199A double mutation was introduced into pHINB1-6His using the Transformer site-directed mutagenesis kit (CLONTECH) and primers GTE-MluI/ HindIII (5'-GAGGCTCCAAGGTTGATGATC-3') and HDINB-DE → AA (5'-CCTAGGTCTTGTCGACCTTGTCG-3'), the latter introducing a PstI restriction site (underlined) to give pHINB1mut-6His. The BamHI fragments from pHINB1C-6His and pHINB1mut-6His were cloned into the same site of pAcG2T to give pAcG2T/HINB1C-6His and pAcG2T/HINB1mut-6His.

The GST-co-transfected SF9 cells with either equivalo DNA using a BaculoGold transfection kit (Pharmingen). Expression of both wild-type and mutant GST/polk was assayed by immunoblotting with anti-GST antisera. Two rounds of amplification produced a high titer stock of recombinant virus expressing GST/polk. The multiplicity of infection yielding optimal expression of full-length fusion proteins was determined empirically.

Purification of GST/polk—Approximately 1 × 10⁶ virus-infected SF9 cells were harvested 3 days after infection and lysed by 20 ml of Lysis Buffer I (1% Triton X-100, 10 mM Tris-HCl, pH 7.5, 10 mM NaHPO₄, pH 7.5, 1 mM EDTA, 5 mM β-mercaptoethanol, 1 × protease inhibitors) on ice for 10 min. Insoluble material was removed by centrifugation to give the cytoplasmic extract. The pellet was resuspended in 20 ml of Lysis Buffer II containing 500 mM NaCl, and incubated on ice for 10 min. Insoluble material was removed by centrifugation to generate nuclear extract. The nuclear extract was diluted 2-fold and bound in batch to 500 μl of glutathione-agarose for 2 h at 4 °C. The resin was harvested by centrifugation and most of the supernatant removed. The resin was resuspended in the remaining supernatant and transferred to a 10-ml disposable column (Bio-Rad) to collect the resin by gravity. The resin was washed with 5 ml of Lysis Buffer II containing 250 mM NaCl, followed by 5 ml of Wash Buffer II (10% glycerol, 100 mM NaCl, 20 mM Tris-HCl, pH 7.5, 0.01% IPEPA-630, 5 mM β-mercaptoethanol, 1 × protease inhibitors). Bound protein was eluted with 3.5 ml of Wash Buffer II containing 10 mM reduced glutathione, and collected in a total of 10 fractions of 350 μl each. GST/polk-containing fractions (determined by SDS-PAGE and immunoblotting) were aliquoted, frozen and stored at -70°C. Insoluble material was removed by centrifugation to generate multiple rounds of freezing and thawing. The mutant fusion proteins were expressed and purified by the same procedure.

DNA Substrates—The oligonucleotide derived primer-templates used as substrates in the DNA polymerase assays (24/44, 27/44, 30/44, and 31/44) were the same as those described by Wagger et al. (6). The 5'/3'-primer-template consisted of oligonucleotides P4-0X-RS (5'-GAATTCCTGACGCCGAGAT3') and T1-OX-WT (5'-ATTCAGACGTCATATACACCGGTTGACGTCATCTGSCAGACGAT3'). Primers were purified by denaturing polyacrylamide gel electrophoresis (PAGE). Five pmol of each primer was 5'-end labeled with T4 polynucleotide kinase (New England Biolabs) in the presence of [γ-³²P]ATP and purified on Bio-Gel P2 (Bio-Rad) spun columns equilibrated in STE (100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The various labeled primers (100 μM) were annealed to the template in a ratio of 1:1.5 (primer-template) by heating to ~95°C for 5 min followed by slow cooling to room temperature.

Preparation of AAF-DNA Template—A synthetic DNA oligo of the sequence 5′-TCTTTGCGGTTCCTT-3′ (site of modification underlined) was purified by denaturing PAGE and desalted using a Sep-Pak C₈ cartridge (Waters, manufacturer’s instructions). 0.38 μmol of purified DNA was dissolved in TE buffer (45 mM Tris-HCl, 1 mM NaCl, pH 8.0) to a concentration of 0.2 mM and incubated with AAAF (1 mM, 10% ethanol, 37°C, 12 h). Adduct DNA was digested with amylase and DNA was eluted by heating at 95°C for 5 min followed by slow cooling to room temperature.
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RESULTS

**Human DinB1 Protein Is a DNA Polymerase, polk**—To determine whether the product of the human **DINB1** gene is a DNA polymerase, we expressed and purified recombinant human DinB1 protein. Expression in both *E. coli* and the yeast *Schizosaccharomyces pombe* consistently resulted in low yields and/or degraded protein (data not shown). However, we were able to express full-length hDinB1 protein fused to GST in insect cells using a baculovirus expression system. The recombinant GST/hDinB1 protein was purified to near physical homogeneity from nuclear extracts by affinity chromatography on glutathione-agarose (Fig. 1A, lane 1). The purified GST/hDinB1 fraction contained primarily full-length fusion protein; however, some degradation products, including free GST, were observed and confirmed by immunoblotting with anti-GST antisera (data not shown).

To test for DNA polymerase activity, various 5'-32P-end labeled oligonucleotide primers were annealed to a 44-nucleotide template and used as substrates. In the presence of dNTPs and Mg2+, the Klenow fragment of *E. coli* DNA polymerase 1 efficiently extended the primer to generate the expected 44-nucleotide product (Fig. 1A, lane 1). The purified GST/hDinB1 fraction contained primarily full-length fusion protein; however, some degradation products, including free GST, were observed and confirmed by immunoblotting with anti-GST antisera (data not shown).

As a control, a GST/hDinB1 mutant protein in which the conserved amino acid residues Asp198 and Glu199 were changed to alanine, was purified by the same procedure (Fig. 1A, lane 2) and shown to be devoid of detectable DNA polymerase activity (Fig. 1C, lanes 3–6), indicating that the observed polymerase activity is intrinsic to the human DinB1 protein. In addition a truncated GST/hDinB1 fusion protein lacking 360 amino acids at the C terminus (GST/hDinB1ΔC) did not demonstrate DNA polymerase activity, indicating that sequences within this less highly conserved portion of the protein are required for activity (data not shown).

We performed a series of experiments to determine the optimal conditions for polk DNA polymerase activity in vitro. As shown in Fig. 2A, between pH 6.5 and 8.5 GST/polk was most active over the range 6.5–7.5 (Fig. 2A, lanes 1–5), with reactions carried out at 37 °C (Fig. 2A, lanes 6–9). To investigate the effect of ionic strength on DNA synthesis, increasing amounts of NaCl were added to the reactions (Fig. 2A, lanes 11–16). GST/polk activity was relatively insensitive to NaCl concentration up to 50 mM, but was significantly inhibited at salt concentrations of 100 mM or higher. As expected, a metal cofactor was required for activity (Fig. 2B). Either Mg2+ or Mn2+ could be utilized, with the former being preferred (Fig. 2B, compare lanes 2 and 3). Based on these observations, all standard DNA polymerase assays using GST/polk were performed at pH 7.0 and 37 °C in the presence of Mg2+.

The range of incomplete extension products produced by GST/polk in the experiments described above suggested that human polk is endowed with limited processivity, as has also been observed for the *E. coli* DinB protein (6, 7). We therefore examined whether purified human PCNA, a sliding clamp known to stimulate the processivity of the replicative DNA polymerases polδ and polε (18–20), increases the extent of DNA synthesis by GST/polk. The ability of PCNA to stimulate the activity of polk on short oligonucleotide-derived primer-templates has been observed previously (21). As shown in Fig. 3, addition of recombinant human PCNA had no detectable effect on GST/polk activity (Fig. 3, lanes 1–4) but could readily stimulate synthesis of full-length products by purified polδ (Fig. 3, lanes 5–7) on a slightly longer template. As was observed on the shorter template, no effect of PCNA on polk activity was observed on the longer template nor did the PCNA possess polymerase activity of its own (data not shown). In contrast to polk, *E. coli* pol IV is stimulated 3000-fold by addition of the bacterial sliding clamp βγ complex (7). A comprehensive study of the effects on replication factors RPA, RFC, and PCNA on polk is currently underway.

**polk Is a Template-directed DNA Polymerase Lacking 3′ → 5′”

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**FIG. 1.** Purification and DNA polymerase activity of wild-type and mutant GST/hDinB1 (polk). A, 50 ng of either wild-type (lane 1) or D198A/E199A double mutant (lane 2) GST/polk glutathione-agarose fractions were analyzed on a 7.5% SDS-polyacrylamide gel and visualized by silver staining. Full-length GST/polk is indicated by an arrow. The positions of molecular weight markers are indicated at the left. B, GST/polk has DNA polymerase activity. The indicated amounts of GST/polk were assayed for DNA polymerase activity at 30 °C on the 25/44 primer-template (lanes 1–3). A DNA polymerase reaction using 5 units of the Klenow fragment of *E. coli* DNA pol I (exo-) was performed as a positive control and is shown in lane 4. The position of the expected full-length product (44 nucleotides) is indicated by an arrow. C, the indicated amounts of either wild-type (lane 2) or mutant (lanes 3–6) were assayed for DNA polymerase activity as described in B.
Proofreading Exonuclease Activity—To demonstrate that GST/polk is a template-directed DNA polymerase we performed polymerase assays in the presence of single deoxyribonucleoside triphosphates (dNTPs) on 4 different primer-templates, each designed to test for the correct incorporation of a particular dNTP. As shown in Fig. 4A, under the single set of conditions tested GST/polk preferentially incorporated the correct nucleotide on each template. However, in all cases significant levels of misincorporation were also observed. For example, on the 27/44 primer-template GST/polk primarily catalyzed the accurate incorporation of dGTP as the first nucleotide, but also supported misincorporation of dATP and to a lesser extent dCTP (Fig. 4A, lanes 7–11). It can also be observed from Fig. 4A that the level of GST/polk activity on the 24/44 substrate was significantly and consistently lower than on the other primer-templates. The cause of this phenomenon is presently unclear, but is most likely related to the immediate sequence context and is apparently exacerbated by decreasing the dNTP concentration from 100 mM to 10 mM (compare Fig. 3A, lane 2, and Fig. 4A, lane 6). Poor extension of this primer-template by E. coli pol IV was also observed by Wagner et al. (6).

Given the detectable levels of nucleotide misincorporation observed in Fig. 4A, we tested GST/polk for 3' → 5' proofreading exonuclease activity. Using a substrate in which the 3' mispaired 31/44 primer-template in the absence (lanes 2–4) or presence of dNTPs (lanes 6–8). Reactions contained 2 nM GST/polk or 5 units of Klenow where indicated. Lanes 1 and 5 contained no added protein. The partial sequence of each primer-template is shown at the top of each panel. For panel B, the size of the expected full-length product (44 nucleotides) produced by Klenow (exo+) is indicated by an arrow.
nucleotide of the primer was not base paired with the template, no shortening of the primer by GST/polk or Klenow (exo-) enzyme was observed in the absence of dNTPs (Fig. 4B, lanes 2 and 3). In contrast, Klenow (exo+) enzyme readily cleaved the primer (Fig. 4B, lane 4). In the presence of dNTPs, the primer could be efficiently extended by Klenow (exo+) enzyme only following cleavage of the mispaired base (Fig. 4B, lane 8). Limited extension by GST/polk was also observed from the 3’ mispaired primer (Fig. 4B, lane 6). The low level of primer extended by Klenow (exo+) enzyme yielded a product 45 nucleotides in length due to incorporation of an additional dATP in a template-independent fashion (22). This nucleotide is normally removed by the 3’ → 5’ exonuclease activity of Klenow (exo+) enzyme. The detectable levels of misincorporation together with the observed lack of a proofreading exonuclease activity suggest that polk is endowed with a low level of fidelity during synthesis of DNA.

To complete our preliminary characterization of GST/polk we tested the sensitivity of the enzyme to aphidicolin and dideoxynucleoside triphosphates (ddNTPs). Aphidicolin is an inhibitor of eukaryotic DNA polymerases α, δ, and ε while polβ and -γ are sensitive to ddTTP (23). In the presence of 25 μM dNTPs GST/pol activity was not inhibited by either aphidicolin (50 ng/μl) or up to an 8-fold molar excess of ddNTPs (data not shown). The lack of sensitivity of polk to aphidicolin and ddNTPs is similar to that observed for human polη (24).

polk Cannot Bypass a Cisplatin Adduct but Can Bypass an AAF Lesion in a Potentially Error-prone Manner—A number of the DNA polymerases in the UmuC/DinB superfamily have been implicated in DNA damage-induced mutagenesis and translesion synthesis (2). We asked whether GST/polk is able to bypass cisplatin (Fig. 5) or an AAF adduct (Fig. 6), lesions that have previously been shown to strongly block other DNA polymerases (reviewed in Ref. 25). GST/polk is unable to bypass d(GpG-N7(1)-N7(2)) cisplatin intrastrand cross-links, even when a 2-fold molar excess of enzyme is added to the primer-template (Fig. 5, lanes 6–9). Klenow (exo-) enzyme is also unable to bypass this lesion (Fig. 5, lane 10), unless high concentrations of enzyme are added (data not shown). Both GST/polk and Klenow (exo-) enzyme arrest one nucleotide prior to the lesion, suggesting that neither enzyme is able to efficiently insert nucleotides across from the damaged bases.

In contrast to the results observed with cisplatin, GST/polk does appear to have an intrinsic ability to bypass N -(deoxyguanosin-8-yl)-N'-2-acetylaminofluorene (G-AAF) adducts (Fig. 6A, lanes 6–9). The bypass appears to be relatively inefficient under the conditions used in these experiments, since relatively high enzyme concentrations are necessary to achieve bypass. Furthermore, even under conditions where bypass occurs, a large percentage of the primer extension products appear to terminate at the site of the AAF lesion. Since it is clear that the GST/polk enzyme is able to insert nucleotides across from the AAF lesion, we were interested in determining which nucleotides could be incorporated. As shown in Fig. 6B, GST/polk primarily inserts dCTP across from an undamaged G residue (lanes 2–5). In contrast, GST/polk appears equally able to incorporate either dTTP or dCTP across from the G-AAF lesion (Fig. 6B, lanes 8–11). A darker exposure of this experiment shows that dATP can also be incorporated across from the G-AAF lesion at much lower frequency (data not shown). These results suggest that polk may play a role in bypass of AAF lesions in vivo and that such bypass could potentially be mutagenic.

**DISCUSSION**

In this study we report that the product of the human DINB1 gene is a DNA polymerase, polk. While this work was in progress, a GST/DinB1 fusion protein purified from the yeast Saccharomyces cerevisiae was reported to have DNA polymerase activity (26). These authors designated human DinB1 protein as DNA polymerase θ (polθ). In view of the fact that this name has been assigned to the product of another gene (27), we suggest that the DinB1 gene product be referred to as polk, a designation approved by the Human Genome Organization nomenclature committee.

The loss of DNA polymerase activity of polk associated with mutation of two highly conserved residues known to be required for the activity of both E. coli pol IV and yeast polθ (6, 28) eliminates the possibility that the activity of our purified GST/polk fraction is due to the presence of a contaminant or interacting protein. We can also conclude that fusion of polk to GST does not abolish its DNA polymerase activity. However, we cannot exclude the possibility that the GST domain alters the DNA polymerase activity of polk in a more subtle way. Polk represents the tenth eukaryotic polymerase reported, and the seventh member of the UmuC/DinB superfamily of proteins endowed with such activity. It is therefore likely that most if not all members of this superfamily are DNA polymerases or nucleotidyl transferases.

To demonstrate DNA polymerase activity we used a simple primer extension assay. Interestingly the largest extension product produced by polk is a single nucleotide shorter than the template. This phenomenon was consistently observed on templates used in the present studies regardless of the primer (e.g. Figs. 1B and 4B). Similar observations have been made using
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other templates of varying length and sequence composition\(^2\) and were observed independently by Johnson et al. (26). The incomplete extension products produced by polk could be extended by the Klenow fragment of E. coli DNA polymerase I, demonstrating that failure to replicate precisely to the end of a linear DNA template is likely an intrinsic property of polk rather than the result of template slippage.\(^2\) Conceivably the enzyme requires interactions with sequences downstream to those located at the active site in order to form a stable protein-DNA complex. The ability of polk to fill in short single-stranded gaps is currently being investigated.

In most of the GST/polk polymerase assays the reaction products consisted of a ladder of primer extension products, ranging from the addition of a single nucleotide to 1 nucleotide short of full-length. A similar size range of products was observed at high GST/polk dilutions where the primer-template was present in vast excess (29). These results suggest that the processivity of polk is variably low to moderate, but not entirely distributive as is the case for E. coli DNA pol IV (6, 7). Similarly, the apparent high rate of nucleotide misincorporation (Fig. 4A) and lack of 3' \(\rightarrow\) 5' proofreading exonuclease activity (Fig. 4B) suggest that polk possesses low fidelity. These conclusions are supported by the results of independent experiments that quantitatively measured the fidelity and processivity of polk in vitro (29).

As noted above, E. coli DNA pol IV is a specialized DNA polymerase that is regulated by the SOS system, suggesting a role in DNA replication and spontaneous mutagenesis in response to certain cellular stress conditions. The apparent low fidelity and moderate processivity of human DNA polk are consistent with a similar role in human cells. The demonstration that the human DINB1 gene encodes polk supports a model in which the role of this polymerase is to facilitate the replication of abnormal templates which inhibit the activity of replicative DNA polymerases polα, polδ, and/or polε.

Abnormal templates might contain particular types of DNA damage or possess aberrant structures. Efficient translesion synthesis by DNA pol IV on templates containing an abasic site, thymine dimer, or 6-4 photoproduct has not been observed (7). However, the bacterial protein can extend misaligned primer-templates resulting in single nucleotide deletions (6). Similarly, a human GST/polk fusion protein is unable to bypass abasic sites, cis-syn thymine-thymine dimers or 6-4 photoproducts in vitro (26).

In this study we have shown that human polk is also unable to bypass a cisplatin lesion. The enzyme is, however, able to inefficiently bypass an AAF-adduct and incorporates primarily either dTTP or dCTP across from the lesion. This result suggests that polk has the potential to bypass AAF by an error-prone mechanism and therefore that polk might play a role in targeted mutagenesis. However, the characterization of translesion synthesis using simple primer extension assays in vitro requires caution. A number of experimental parameters may influence the outcome, including reaction conditions, enzyme concentration, nucleotide concentration, and template sequence context (25). The physiological relevance of the bypass of AAF lesions by polk is uncertain since it is observed only at high enzyme concentrations, and even under these conditions a significant fraction of the enzyme is arrested at the site of the lesion. However, it remains a formal possibility that polk can be utilized in human cells for translesion synthesis of specific lesions (such as AAF or other types of DNA damage which have not yet been tested), as may be the case for pol\(\eta\) (30).

In human cells polk may function in additional or alternative modes other than the replication of abnormal templates. For example, the enzyme may be required to replicate specific normal template regions in an abnormal (error-prone) manner, such as appears to be required during somatic hypermutation of immunoglobulin genes (31). More precise definition of the biological function(s) of this enzyme will require further detailed biochemical and genetic studies, including the phenotypic characterization of mammalian cells defective in this enzyme activity.

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\(^2\) V. L. Gerlach, W. J. Feaver, and E. C. Friedberg, unpublished data.

Fig. 6. GST/polk displays inefficient, potentially error-prone bypass of an AAF lesion. A. replication of 5 nM wild-type (lanes 2–4) or AAF-containing (lanes 7–9) primer-templates was tested using the indicated concentrations of GST/polk. Reactions were for 10 min at 37 °C. 1 nM Klenow (exo\(^-\)) enzyme was used as a control (lanes 5 and 10). The position of the G-AAF adduct is indicated at the right and is located at the 30 nucleotide (nt) position. The unextended running start primer (lanes 1 and 6) is 20 nucleotides and the full-length extension product is 53 nucleotides in length. B, specificity of nucleotide incorporation by GST/polk across from undamaged G (left) or G-AAF (right). Nucleotide incorporation was tested using 2.5 nM GST/polk and either 100 \(\mu\)M individual dNTPs (lanes 2–5 and 8–11) or a 100 \(\mu\)M pool of all four dNTPs (lanes 6 and 12) for 10 min at 37 °C. Reactions lacking GST/polk contained all four dNTPs (lanes 1 and 7). A standing start primer of 29 nucleotides was used; the first nucleotide incorporated is directly across from the G-AAF lesion (or undamaged control).
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