129-derived Strains of Mice Are Deficient in DNA Polymerase \( \tau \) and Have Normal Immunoglobulin Hypermutation

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Abstract

Recent studies suggest that DNA polymerase \( \eta \) (pol\( \eta \)) and DNA polymerase \( \tau \) (pol\( \tau \)) are involved in somatic hypermutation of immunoglobulin variable genes. To test the role of pol\( \tau \) in generating mutations in an animal model, we first characterized the biochemical properties of murine pol\( \tau \). Like its human counterpart, murine pol\( \tau \) is extremely error-prone when catalyzing synthesis on a variety of DNA templates in vitro. Interestingly, when filling in a 1 base-pair gap, DNA synthesis and subsequent strand displacement was greatest in the presence of both pols \( \eta \) and \( \tau \). Genomic sequence analysis of Pol\( \tau \) led to the serendipitous discovery that 129-derived strains of mice have a nonsense codon mutation in exon 2 that abrogates production of pol\( \tau \). Analysis of hypermutation in variable genes from 129/SvJ (Poli\( \tau \)/Poli\( \eta \)) and C57BL/6J (Poli\( \tau \)/Poli\( \eta \)) mice revealed that the overall frequency and spectrum of mutation were normal in pol\( \tau \)-deficient mice. Thus, either pol\( \tau \) does not participate in hypermutation, or its role is nonessential and can be readily assumed by another low-fidelity polymerase.

Key words: genomic organization • immunoglobulin variable genes • DNA polymerase \( \eta \) • cytosine deamination • base excision repair

Introduction

The recent discovery of the \( Y \)-family of DNA polymerases (1) has provoked much investigation of their biochemical properties when they replicate damaged and undamaged DNA templates (2–5). One of the better characterized members of this large family is pol\( \eta \). Both \textit{Saccharomyces cerevisiae} and human orthologs of pol\( \eta \) can efficiently synthesize past various types of DNA damage (6–9), because their active sites are more accessible to solvents than that of a high fidelity polymerase (10). Humans with defects in their \textit{POLH} gene are afflicted with the xeroderma pigmentosum variant (XP-V) phenotype, which is characterized by an increased sensitivity to ultraviolet light and sunlight-induced skin cancers (11). Thus, pol\( \eta \) has an important biological role in protecting us from the deleterious effects of sunlight. Furthermore, as a result of its nonrestrictive catalytic site, pol\( \eta \) misincorporates nucleotides on undamaged templates (12–14). Pol\( \eta \) has been implicated in somatic hypermutation of antibodies, as immunoglobulin variable (V) genes from XP-V patients have an altered spectrum of mutations (15, 16), which is consistent with the types of mutations generated by pol\( \eta \) in vitro (17, 18).

In addition to pol\( \eta \), humans possess three other \( Y \)-family polymerases, pols \( \tau \), \( \kappa \), and Rev1, with pol\( \tau \) being the most
closely related to pol\(\eta\) (19). Human pol\(\gamma\) has a limited ability to replicate past DNA lesions in vitro, suggesting that it is not as efficient as pol\(\eta\) in translesion synthesis (20, 21). However, unlike pol\(\eta\), pol\(\gamma\) possesses a 5’ dRP lyase activity and may participate in a specialized form of base excision repair (22). Perhaps the most striking property of pol\(\gamma\) in vitro is its infidelity when replicating undamaged DNA. It is most error-prone when copying template T, where the enzyme misincorporates dGMP by 3- to 10-fold over the correct Watson and Crick base, dAMP (22–26). The biological function of pol remains unknown, and no human disease or repair-related deficiency has been directly linked to mutations in the POLI gene. Because of its extreme low-fidelity in copying undamaged DNA, pol\(\gamma\) is a good candidate for introducing mutations into V genes (23, 27). Indeed, during the course of our studies, Faili et al. (28) reported that a human Burkitt lymphoma cell line (BL2), with a homozygous deletion of both POLI alleles, is deficient in somatic hypermutation. As the first step toward understanding the biological function of pol in a whole-animal system, we assayed the frequency and pattern of mutations in V regions from the heavy chain locus in pol\(\gamma\)-deficient mice and compared them to similar data from pol\(\gamma\)-proficient mice.

### Materials and Methods

**Overexpression and Purification of Mouse Pol and Pol\(\gamma\).** Cloning of the full-length mouse Pol\(\gamma\) cDNA in plasmid pJM297, has been described previously (19). An ~2.8-kb Ncol to Pol fragment from pJM297 was subcloned into the baculovirus expression vector pJM296 (23) that had been digested with Ncol and SmaI, to create the GST-Poli fusion construct, pJM306. GST-tagged mouse pol\(\gamma\) was overexpressed in SF9 insect cells infected with pJM306–derived baculovirus, and subsequently purified by Glutathione–agarose affinity chromatography and hydroxyapatite chromatography as previously described for the GST-tagged human pol\(\eta\) (23). Histidine-tagged murine pol\(\eta\) was overproduced and purified as described previously (29).

**DNA Templates.** The synthetic oligonucleotides used as primers or templates in the in vitro replication assays were synthesized by Lofstrand Laboratories using standard techniques and were gel-purified before use. The sequence of each oligonucleotide is given in the legend of each figure. Primers were 5’-labeled with \(\gamma^{32}\text{P}\)ATP (5000 Ci/mmol; 1 Ci = 37 GBq; American Biosciences) using T4 polynucleotide kinase (Invitrogen).

**Replication Reactions.** Radiolabeled primer-template DNA were prepared by annealing the 5’-32P-labeled primer to the unlabeled template DNA at a molar ratio of 1:1.5. For gapped templates, a second unlabeled oligonucleotide was also annealed to the template in a ratio of 1:2. Standard replication reactions (10 \(\mu\)l) contained 40 mM Tris•HCl pH 8.0, 5 mM MgCl\(_2\), 100 \(\mu\)M of each ultrapure dNTP (Amersham Biosciences), 10 \(\mu\)M \(\beta\)-mercaptoethanol, 250 \(\mu\)g/ml BSA, 2.5% glycerol, 10 nM 5’-[32P] primer-template DNA, and 3 nM GST-Poli. Reactions were incubated at 37°C for 30 min (unless noted otherwise), and reactions were terminated by the addition of 10 \(\mu\)l of 95% formamide/10 mM EDTA containing 0.1% xylene cyanol and 0.1% Bromophenol blue. Samples were heated to 100°C for 5 min and 5 \(\mu\)l of the reaction mixture was added to 20% polyacrylamide/7 M urea gels and separated by electrophoresis. Replication products were subsequently visualized by PhosphorImager analysis (FujiFilm Software Inc.).

**Identification and Genomic Sequence of a Mouse Pol BAC Clone.** A BAC clone, 12337 (Incyte Genomics), containing ~100 to 120 kb of DNA encompassing the mouse Poli genomic region was isolated by screening a BAC clone library using a mouse Poli EST (GenBank/EMBL/DDBJ accession no. AA162008) as a probe. The 12337 BAC clone was then subjected to random “shotgun” sequencing (NHGRI, NIH, Bethesda, MD). An approximate 36 kb DNA sequence of the mouse genome encompassing Poli has been deposited in GenBank and assigned the accession nos. AF489425 and AF489426.

**PCR Genotyping of Murine Pol Codon 27.** To identify mutant or wild-type alleles of codon 27, an 88-bp fragment of mouse Poli exon 2 was amplified from mouse genomic DNA using the following forward (5’-CAGTTTGCAGTCAAGGGCC) and reverse (5’TGACCTGGGGCATAAAGGC) primers. PCR amplifications were performed in a 20 \(\mu\)l reaction using AmpliTaq DNA polymerase (PE Biosystems) and 100–200 ng of genomic DNA with standard reaction conditions as suggested by the manufacturer. The reaction was performed for 45 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min. A 10 \(\mu\)l aliquot from the completed PCR reaction was then treated with TaqI and incubated at 65°C for 1 h. The TaqI-treated and untreated portions of the PCR reaction were separated on a 6% polyacrylamide gel (Invitrogen), and stained with ethidium bromide. Genomic DNAs from various strains of mice were obtained from The Jackson Laboratory (C57BL/6j, 129/J, 129/SvJ, 129/ReJ); Novagen (BALB/c, ICR Swiss); or as a generous gift from Gilbertus van der Host (Erasmus University, Rotterdam, The Netherlands; 129/Ola).

**Tests Exacts and Western Blot Analysis.** Testis tissues from various 129 or C57BL/6 mice were provided as a generous gift by Eric Wawrousek (National Eye Institute, NIH, Bethesda, MD) or purchased from The Jackson Laboratory. Protein extracts from mouse testis were prepared from frozen whole testes. The testes were thawed on ice in lysis buffer consisting of 9 M urea, 4% Nonidet P-40, 2% biolyte ampholyte 5/8 (Bio-Rad Laboratories), 2% 2-mercaptoethanol, and 1× Complete EDTA-free protease inhibitor cocktail (Roche). Thawed testes were first minced and additional lysis buffer added to a final ratio of 8 ml to 1 g of tissue. The testis tissue was homogenized three times for 10–15 s on ice using a polytron PT3000 (Brinkmann Instruments) and the homogenate was then centrifuged for 1 h at 100,000 g at 22°C. The supernatant was removed and frozen on dry ice. Total protein concentration was determined by using the Bradford Protein assay dye reagent concentrate (Bio-Rad Laboratories). For Western blot analysis, ~40 to 50 \(\mu\)g of total protein was subjected to electrophoresis in SDS-10% PAGE gels. Proteins were electro-transferred to an Immobilon P membrane (Millipore) and subsequently probed with a 1:5,000 dilution of pol polyclonal rabbit antibody raised against a 16-mer peptide (AEWERAGAARPSAHRC) corresponding to the very COOH terminus of murine pol that had been conjugated to Keyhole limpet hemocyanin antigen (Covance). Levels of mouse actin were used as protein loading controls and were detected using an anti-actin antibody (Sigma-Aldrich). Mouse pol and actin proteins were subsequently visualized using the CSPD-chemiluminescent “Western Light” chemiluminescent assay (Applied Biosystems).

**Mice Used for Hypermutation Studies.** 129/SvJ and C57BL/6J mice were purchased from The Jackson Laboratory and used at
Results

Fidelity of Murine Pol Replication on Various DNA Templates. To determine the biochemical properties of murine pol, the enzyme was overproduced and purified as a recombinant GST-tagged protein from baculovirus-infected insect cells. Similar to our studies with human pol (23, 27), murine pol appears to be extremely error-prone. When replicating a recessed template, pol readily misincorporates dNMPs opposite most template bases, but does not put in dCMP opposite T (Fig. 1A). When replicating the end of a template, murine pol is unable to misincorporate dGMP or dAMP opposite G, yet clearly favors the misincorporation of dCMP opposite template C (Fig. 1B).

Strand Displacement by Polκ- and Polη during Gap Filling. Recent studies suggest that an early event in somatic hypermutation is the deamination of cytosines in DNA (30). It is hypothesized that the resulting uracil moiety gives rise to C→T transitions if copied by a high-fidelity polymerase, or both transitions and transversions if uracil is removed by uracil DNA glycosylase to produce an abasic site which could be copied by an error-prone polymerase (30, 31). Alternatively, the abasic site could be further processed by base excision repair proteins into a single nucleotide gap (5, 32). As polκ possesses intrinsic 5′ dRDP lyase activity (22) and shows enhanced catalytic activity on gapped substrates (22, 27), it seems reasonable to think that polκ might participate in such a pathway. Because polη has previously been implicated as being the A−T mutator in hypermutation (15, 18), we were interested in assaying the efficiency and accuracy of both polymerases in replicating a 1 base-pair gap. The template base chosen for the gap-filling reaction was dG, as such a substrate would be expected to occur through the deamination of the complementary dC. As seen in Fig. 2, polκ is active and error-prone on this substrate. In the presence of all four dNTPs, there is very limited strand displacement with 1–2 bases inserted at the original 1 bp gap. The correct base dCMP is preferentially inserted opposite template G, but dAMP and dTMP are also efficiently misinserted. Under the same conditions, polη is more accurate than polκ, with the predominant insertion of the correct base, dCMP, and very little misinsertion of dAMP, dGMP, or dTMP. Both polymerases appear to replicate the 1 bp gap with roughly the same efficiency, but polη is clearly better at strand displacement than polκ, and some primers are even fully-extended to the end of the template. When the two polymerases are added together, the major product of the 1 bp gap-filling assay is actually 3 bp long, and there appears to be significantly more strand displacement in the presence of both polymerases than in the presence of either polκ or polη alone. Thus, both polκ and polη can participate in a 1 bp gap-filling reaction, and their combined actions would lead to significant strand displacement.

Genomic Structure of Murine Pol. To achieve our initial goal of generating a polκ-deficient mouse, we determined the entire genomic structure of the Poli gene located on a
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Figure 2. DNA synthesis by murine pol and polη on a 1 bp gapped substrate. The sequence of the gapped substrate is shown above the gel. The extent of murine pol- or polη-dependent (mis)incorporation was measured at each template site in the absence of dNTPs (0), all four dNTPs (4), or each individual dNTP (G, A, T, C). Pr. = radiolabeled primer. Reactions containing pol or polη alone, or together, lasted for 20 min at 37°C. From these experiments, one can see that pol is more error-prone than polη when replicating a template G in a gapped substrate and that when combined, the major products of the 1 bp gap-filling reaction are 3 bp and longer.

120-kb mouse genomic BAC clone, 12337. The murine Poli gene was compared to the human gene, based on the human genomic sequence (GenBank/EMBL/DDBJ accession no.: NT_010893). Similar to human POLI, the mouse Poli gene has 10 exons (Fig. 3). The gene spans a region of ~23 kb on mouse chromosome 18, band E2, that shares synteny with human chromosome 18. Furthermore, the positions of introns and the sizes of exons are highly conserved between mouse and human genes. Intron 9 of the mouse gene has not been completely sequenced due to the presence of a mouse L1 retrotransposon sequence, which was detected by BLAST homology searches using the sequence of the 5’ and 3’ ends of the intron.

129-derived Strains of Mice Have a Nonsense Mutation in Their Poli Gene. Sequence analysis of the 12337 BAC clone revealed a single nucleotide polymorphism in exon 2 which changed the wild-type Serine 27 codon, TCG, to an amber stop codon, TAG. As the clone came from a 129/SvJ mouse library, we determined if the mutation was present in other 129-derived strains. Fortuitously, the mutation can be easily detected, as it destroys a TaqI restriction site in the wild-type exon 2 (TCGA; wild-type serine codon 27 underlined). Using a PCR-based assay, we were able to genotype mouse strains for the presence or absence of the polymorphism. PCR products encompassing codon 27 from all of the mouse 129 strains analyzed (129/J, 129/SvJ, 129/ReJ, and 129/Ola) were undigested by the TaqI enzyme, indicating that they were homozygous for the nonsense mutation (Fig. 4 A). In contrast, TaqI digestion of the PCR product from C57BL/6j genomic DNA resulted in complete cleavage, showing that this strain is homozygous for the wild-type codon. Genotypic analysis of several other laboratory strains of mice such as BALB/c and ICR Swiss showed that they also had the wild-type codon for Pol (unpublished data).

In theory, the nonsense mutation should result in a pol protein consisting of just 26 amino acids. This truncation occurs well before any of the conserved polymerase domains of pol and should result in a complete loss of pol activity within the cell. To confirm that the mutation abrogated pol synthesis, the level of pol was analyzed by Western blotting of testis extracts, where pol was normally highly expressed (19, 27). Testis extracts from 129/SvJ and C57BL/6j mice were separated by gel electrophoresis, and pol was detected using an antibody raised against a peptide corresponding to the very COOH terminus of murine pol. This antibody recognizes full-length or mis-spliced variants containing the catalytic domain of pol. As shown in Fig. 4 B, extracts from C57BL/6j mice had significant levels of pol protein, whereas extracts from 129/SvJ mice had no detectable levels of full-length protein. Thus, the S27 nonsense mutation leads to a severe, if not total, deficiency in pol protein in 129 mice.

Hypermutation of Immunoglobulin Genes in the Absence of Poli. The availability of mice naturally deficient in pol gave us an opportunity to determine if pol is required for somatic hypermutation of immunoglobulin genes. Mutations were identified in the intron region downstream of rearranged V genes on the heavy chain locus from both 129/SvJ and C57BL/6j mice. All the mutations in clones with different V rearrangements were counted, and only unique mutations in clones with the same rearrangements
For the 129/SvJ clones, 98% of the mutations were base substitutions, whereas the C57BL/6 mice were not immunized. The frequency was probably higher in the 129/SvJ clones, and 0.5% mutations/bp for C57BL/6J clones. All frequency of mutation was 2.5% mutations/bp for 129/SvJ clones were mutated (137 out of 258; Fig. 5 A). The over-}
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were scored. For 129/SvJ mice, 96% of the clones had mutations (81 out of 84); and for C57BL/6J mice, 53% of the clones were mutated (1,37 out of 258; Fig. 5 A). The overall frequency of mutation was 2.5% mutations/bp for 129/SvJ clones, and 0.5% mutations/bp for C57BL/6J clones. The frequency was probably higher in the 129/SvJ clones because the mice were inadvertently immunized before sacrifice, whereas the C57BL/6 mice were not immunized. For the 129/SvJ clones, 98% of the mutations were base substitutions (703 out of 715), and the rest were 9 deletions of 1–2 nucleotides, 1 deletion of 30 nucleotides, and 2 insertions of a single base. For the C57BL/6 clones, 99% of the mutations were substitutions (452 out of 454); the rest were a deletion of 1 nucleotide and an insertion of 44 nucleotides. Thus, there were very few insertions or deletions in either strain, with the vast majority of mutations being base substitutions.

The types of base substitutions in the two groups of clones are shown in Table I. For both 129/SvJ and C57BL/6J clones, 56% of the changes were at A and T bases, and 44% were at G and C bases. The percent of individual types of substitutions was very similar between the two groups, with the exception of a decrease of C to T substitutions in the 129/SvJ clones (10%) compared with the C57BL/6 clones (17%), as recorded from the nontranscribed strand (ΔP[x2] = 0.01). The location of mutations in the intron sequence from both strains is diagrammed in Fig. 5 B. Both strains have mutations at DNA motifs that are associated with increased hypermutability, RGYW and WA (mutable positions are underlined; R = A or G, Y = C or T, W = A or T) (17, 33). For example, mutations at G in RGYW or RGYW-like sequences are seen in nucleotide positions 40, 48, and 57; and mutations at A in WA hotspots are found in positions 8, 267, and 325. The figure shows that the overall distribution of mutations is similar between the two strains.

**Discussion**

*129-derived Strains of Mice Lack Pol*.

Like human pol, murine pol exhibits extremely low-fidelity synthesis when copying a variety of undamaged DNA templates. During the course of our attempts to generate a murine knock-out of Pol, we serendipitously discovered that inbred mice derived from the commonly-used 129 strains have a nonsense mutation at the beginning of the Pol gene. The amber codon replaces the Ser27 codon and results in a truncated protein lacking any catalytic function. Similar mutations producing short products have been identified in the human *POLH* gene encoding polβ from XP-V patients. For example, the XP4BE mutation results in a 27 amino acid product, and the XP30RO mutation yields a 42-amino acid fragment (34). Both of these mutations lead to a severe XP-V phenotype which includes increased UV sensitivity, enhanced UV-induced mutations, and an elevated incidence of UV-induced skin cancers indicating that these mutations result in a loss of polβ activity. As the mouse *Pol* mutation produces a similar truncation, it is likely that there is a severe defect in pol function(s) in 129 mice. Indeed, our inability to detect polk in Western blots of testis extracts from 129 mice supports such a conclusion.

As 129 mice have no detectable levels of polk, are there any phenotypes that can be attributed to this deficiency? The fact that 129 mice are viable suggests that polk is not essential for growth. However, although we did not detect polk protein in tissue extracts, it is possible that there is very limited suppression of the amber nonsense codon during
development so as to produce enough protein for viability. Another possibility is that the 129 strain has mutations in other genes that could compensate for the lack of pol. Thus, the nonessential role of pol in development needs to be established genetically in mice with the mutation in a defined wild-type background. Concerning cancers, it has been observed that 129 strains are resistant to gamma radiation-induced thymic lymphomas (35), indicating that cer-
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bp, indicating that robust hypermutation can occur in the
this study, clones from 129/SvJ mice had 2.5% mutations per
clones from both immunized (36, 37) and unimmunized (38,
region contains a high frequency of unselected mutations in
ranged V genes on the heavy chain locus. This 344-base re-
gated cytosines. To test if pol
DNA polymerases during repair and/or replication of deam-
Mutations are likely introduced into V genes by error-prone
and pattern of mutations was studied in 129/SvJ mice. Mu-
Values have been corrected to represent a sequence with equal amounts
of the four nucleotides. All mutations are shown from the
nontranscribed strand. Substitutions at four allelic nucleotides have been
excluded from the comparison.

Table I. Spectra of Mutations in 129/SvJ and C57BL/6J
JH Introns

| Substitution | 129/SvJ (689 mutations) | C57BL/6J (452 mutations) |
|--------------|-------------------------|--------------------------|
|              | %                       | %                        |
| A to:        |                         |                          |
| T            | 15                      | 17                       |
| C            | 10                      | 6                        |
| T to:        |                         |                          |
| C            | 9                       | 11                       |
| A            | 6                       | 8                        |
| G            | 5                       | 4                        |
| G to:        |                         |                          |
| A            | 13                      | 12                       |
| T            | 5                       | 3                        |
| C            | 8                       | 5                        |
| C to:        |                         |                          |
| A            | 3                       | 2                        |
| G            | 4                       | 3                        |

Values have been corrected to represent a sequence with equal amounts
of the four nucleotides. All mutations are shown from the
nontranscribed strand. Substitutions at four allelic nucleotides have been
excluded from the comparison.

tain DNA lesions may be processed more accurately in the
absence of pol.

One note of caution should be mentioned: many investi-
gators use 129-derived embryonic stem cells for gene target-
ing. Although the recombinant mice are back-crossed to wild-type strains, experiments are often performed with F2
crosses that may carry the 129-derived Poli mutation. Indeed, we have analyzed a number of repair-deficient mice gener-
ated with 129 embryonic stem cells, and found that they have mixed +/+ , +/− , and −/− genotypes for Poli (unpublished data). It is therefore possible that any repair phenotype
previously associated with a particular gene knockout may also have been influenced by a deficiency in pol.

Normal V-Gene Hypermutation in Pol-deficient Mice.
Mutations are likely introduced into V genes by error-prone DNA polymerases during repair and/or replication of deam-
inated cytosines. To test if pol is involved, the frequency and pattern of mutations was studied in 129/SvJ mice. Mu-
tations were examined in the JH4 intron region 3’ of rearranged V genes on the heavy chain locus. This 344-base re-
region contains a high frequency of unselected mutations in
clones from both immunized (36, 37) and unimmunized (38,
39) mice, ranging from 0.3% to 1.9% mutations per bp. In
this study, clones from 129/SvJ mice had 2.5% mutations per
bp, indicating that robust hypermutation can occur in the
absence of pol. A normal level of hypermutation in V<sub>k</sub>
genes from 129 mice has also been previously reported (38).
Furthermore, the types and location of bases changes were
similar between pol-deficient and proficient mice.

Based on these observations with the pol-deficient 129 mice, we conclude that either pol does not participate in
hypermutation, or that its role is nonessential and can be readily assumed by another low-fidelity polymerase. These results contrast with those of Faili et al., who found that
hypermutation does not occur in a human BL2 cell line with a homozygous deletion of POLI (28). Such differ-
ences might be due to short-term stimulation of B cells in culture where only pol may be available (40) versus long-
term stimulation in mice where other DNA polymerases can substitute for pol in the hypermutation process.

Although pol-deficient mice undergo normal somatic hypermutation, there are experimental results that suggest
pol helps to facilitate hypermutation. In pol-deficient BL2-cells, there was a reduction in G•C mutations (28),
which suggests that in wild-type cells, pol has access to the site of deaminated cytosines to produce G•C mutations. In
pol-deficient mice, there was a normal frequency of hy-
permutation at G, A, T, and C nucleotides, which is most
likely due to synthesis by polh. While polh appears to be
more accurate than pol in the gap-filling assay shown in
Fig. 2, it is nevertheless a low-fidelity DNA polymerase
(13, 41) and could easily generate mutations opposite all 4
base pairs through limited strand displacement. Interes-
tingly, in polh-deficient humans, hypermutation occurs
mostly at G•C pairs (15), which is consistent with synthesis
by pol at a 1-bp gap and little to no strand displacement (Fig. 2), or replication by pol past an abasic site (24, 42).
Finally, a recent study indicates that pol and polh physis-
cally interact and colocalize in replication foci in the
nucleus (43). As shown here, gap-filling and subsequent
strand displacement is most robust when both enzymes are
added together, and such activities may result in the intro-
duction of multiple mutations located several base pairs
from the initial single nucleotide gap.

Further studies on the precise biological roles of the
enigmatic pol are clearly necessary, and it is hoped that the
wide availability of 129 strains of mice naturally deficient in
pol will facilitate such research.

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Figure 5.

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