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The Tyr-265-to-Cys mutator mutant of DNA polymerase β induces a mutator phenotype in mouse LN12 cells

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ABSTRACT **DNA polymerase β functions in both base excision repair and meiosis. Errors committed by polymerase β during these processes could result in mutations. Using a complementation system, in which rat DNA polymerase β substitutes for DNA polymerase I of *Escherichia coli*, we previously isolated a DNA polymerase β mutant in which Tyr-265 was altered to Cys (Y265C). The Y265C mutant is dominant to wild-type DNA polymerase β and possesses an intrinsic mutator activity. We now have expressed the wild-type DNA polymerase and the Y265C mutator mutant in mouse LN12 cells, which have endogenous DNA polymerase β activity. We demonstrate that expression of the Y265C mutator mutant in the LN12 cells results in an 8-fold increase in the spontaneous mutation frequency of λ*cII* mutants compared with expression of the wild-type protein. Expression of Y265C results in at least a 40-fold increase in the frequency of deletions of three bases or more and a 7-fold increase in point mutations. Our results suggest that the mutations we observe *in vivo* result directly from the action of the mutator polymerase. To our knowledge, this is the first demonstration of a mutator phenotype resulting from expression of a DNA polymerase mutator mutant in mammalian cells. This work raises the possibility that variant polymerases may act in a dominant fashion in human cells, leading to genetic instability and carcinogenesis.**

DNA polymerase β (pol β) is a 39-kDa protein with both nucleotidyltransferase and 5′-deoxyribose phosphodiesterase activities (1, 2). Evidence has been provided for a role for pol β in both base excision repair and meiosis (3, 4). There is no evidence that pol β functions in replication of the mammalian genome but pol β has been shown to participate in DNA replication in *Escherichia coli* in the absence of DNA polymerase I (5). However, mice that are completely deficient in pol β die at 10 days postconception, suggesting that pol β is essential for embryonic development (6). The physiological DNA substrate for pol β is believed to be a small gap because it has been shown that pol β is processive on gaps of 6 bases or less and that the activity and fidelity of pol β are highest on a 1-bp gap with a 5′ phosphate (7, 8). Several studies have suggested that pol β is the least accurate mammalian DNA polymerase [for example, see Kunkel (9)], suggesting that gap-filling synthesis by pol β *in vivo* may result in mutations.

To further our understanding of the cellular role of pol β, we have identified mutants that are dominant to the wild-type pol β (β-WT). One of these mutants, termed pol β-TR, consists only of amino acid residues 1–170, which comprise a DNA-binding domain of the protein (10). The other mutant, Y265C, is altered from Tyr-265 to Cys (10). This mutant was shown to have an intrinsic mutator activity in three different *in vitro* assays (11). Using the herpes simplex virus thymidine kinase (HSV-*tk*) forward mutation assay (12, 13), we found that the Y265C protein commits both base-substitution and frameshift mutations at frequencies increased significantly over β-WT; Y265C also produces multiple mutations on the same template in this assay. Expression of the Y265C mutant in *E. coli* sensitizes the cells to alkylating agents, even in the presence of β-WT protein (10). In *Saccharomyces cerevisiae*, expression of the Y265C mutant also renders the cells sensitive to alkylating agents. In addition, expression of the Y265C protein confers a mutator phenotype to the *S. cerevisiae* cells (14).

To determine whether expression of the Y265C protein in mammalian cells also results in a mutator phenotype and to identify the types of mutations that result from the action of Y265C, we subcloned the Y265C and β-WT cDNAs into the steroid-inducible pGRE5–2 vector (15) and transfected the resulting constructs into mouse LN12 cells (16). These cells carry both the *supF* and λ*cII* transgenes on a λ shuttle vector that has been integrated into the genome as multiple copies; they are also pol β-proficient. After inducing expression of either the β-WT or Y265C protein in the LN12 cells, we rescued the λ shuttle vector and determined the spontaneous mutation frequency resulting from mutations detected in the *cII* gene. We also obtained the DNA sequences of the *cII* mutants. Our data show that expression of Y265C confers a mutator phenotype to the LN12 cells, and the types of errors resulting from its expression *in vivo* are similar to the types of errors it commits *in vitro*. Therefore, our data suggest that the Y265C protein is dominant to the mouse pol β protein *in vivo* and that mutations that arise in the LN12 cells expressing Y265C are most likely committed directly by this enzyme.

MATERIALS AND METHODS

Strains, Cell Lines, and Media. For cloning experiments, we used the *E. coli* DH5αMCR strain with the genotype *mcrA* (*mrr-hsdRMS-mcrBC*) φ80Δ*lacZ*(M15) (*lacZYA-argF*)U169 *deoR recA1 endA1 phoA supE44 thi-1 gyrA96 relA1.* Transformants of DH5αMCR were selected on LB agar containing 50 μg/ml ampicillin. The strains used to obtain the frequency of the λ*cII* mutants were G1217 (nonselecting strain) with the genotype Δ(*mcrA*)183 Δ(*mcrCB-hsdSMR*)173 *endA1 supE44 thi-1 gyrA96 relA1 lacZ supF* and G1225 (*cII* mutant-selecting strain), which is G1217 with *hflA*::Tn5 *hflB29*::Tn10.

Mouse LN12 cells, carrying 100 copies of the λ*supF* shuttle vector integrated into their genome, were employed in the experiments described here (16). This shuttle vector contains the *supF* and the *cII* genes, both of which can be used as targets

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Abbreviations: pol β, DNA polymerase β; β-WT, wild-type pol β; HSV-*tk*, herpes simplex virus thymidine kinase gene; Dex, dexamethasone; HA, hemagglutinin; BER, base excision repair.
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for monitoring mutagenesis. The LN12 cells were maintained in DMEM with 10% calf serum donor (CSD) supplemented with 400 μg/ml G418 (GIBCO/BRL) at 37°C in a humidified incubator with 5% CO_2.

Subcloning of pol β cDNAs into pGRE5–2. To subclone the Y265C mutant and the β-WT cDNAs, we first designed two oligonucleotides, which were synthesized by the Keck Center for Biotechnology at Yale University School of Medicine, to amplify the cDNAs from the pβL plasmid (11). Oligo 1 has the sequence 5′-GGA GCT CGA GCG AAA TGA GCA AAC GCA AG; it contains a *Sac*I site at its 5′ end, and the portion underlined anneals to nucleotides 1–15 of the rat pol β cDNA. Oligo 2 has the sequence 5′-GGGTACC **CTA TGC GTA GTC TGG TAC GTC ATA TGG GTA** TTC GCT CCG GTC CTT GGG TTC CCG GTA G; it carries a *Kpn*I site at its 5′ end, a sequence encoding a hemagglutinin (HA) epitope, shown in bold, and the underlined portion anneals to nucleotides 977-1004 of the rat pol β cDNA. These oligonucleotides were used in a PCR to amplify the β-WT and Y265C rat pol β cDNAs in the following reaction conditions: 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 1 min, and 72°C for 2 min. After amplification, the products were ligated into the pCRII vector (Invitrogen) and transformed into DH5αMCR cells. After confirming by DNA sequencing that the ampicillin-resistant transformants carried either the β-WT or Y265C cDNA, plasmid DNA was isolated and digested with *Sac*I and *Kpn*I and ligated into the pGRE5–2 vector that had been digested with the same restriction endonucleases. The resulting ligation reactions were transformed into DH5αMCR, and plasmid DNA from the ampicillin-resistant transformants was sequenced to be certain that either the β-WT or Y265C cDNA had been subcloned. The pGRE5–2 steroid-inducible vector was a gift from John White (McGill University, Montreal) (15). This vector carries five glucocorticoid-inducing elements in the promoter region, rendering expression of either β-WT or Y265C inducible in the presence of dexamethasone (Dex). The resulting constructs express the rat pol β proteins, fused at their 3′ ends, to the HA epitope. Fusion of the β-WT protein to the HA epitope does not alter the activity of the enzyme (C.A.C. and J.B.S., unpublished data).

Transfection of pol β cDNAs into Mouse LN12 Cells. Transfection of the pol β cDNAs was performed by using SuperFect Transfection Reagent (Qiagen). Briefly, LN12 cells were seeded into 60-mm dishes (approximately 2.0×10^5 cells per dish) and grown overnight in DMEM with 10% CSD containing 400 μg/ml G418 to approximately 40% confluence. Five micrograms of DNA (5.0 μg of either of the pol β cDNAs and 0.05 μg of pTk, which is a plasmid that carries the HSV-*tk* gene) was mixed with cell growth medium containing no serum or antibiotics to a final volume of 150 μl. Twenty microliters of SuperFect Reagent was added to the DNA mixture and incubated at room temperature for 10 min. The cells were washed with PBS, and 1 ml of growth medium containing serum and antibiotics was added to the DNA/SuperFect tube, mixed, and added immediately to the cells. This mixture was incubated at 37°C in a humidified incubator with 5% CO_2 for 2–3 hr. The transfection medium was removed, the cells were washed with PBS, and fresh medium containing 10% CSD and 400 μg/ml G418 was added. After overnight incubation, HAT (hypoxanthine/aminopterin/thymidine) (Sigma) was added to select for cells that contained the pTK plasmid and the pol β cDNA. Individual transfectants were cloned by limiting dilution. Colonies derived from single cells were assayed for the presence of the pol β cDNAs and the *supF* gene by PCR analysis.

Western Blot Analysis of Clones. Colonies that grew in growth medium containing both HAT and G418 were expanded in duplicate in 24-well plates. The cells were treated with 25 nM Dex for 48 hr. Once they had reached confluence, the cells were trypsinized and resuspended in buffer A (50 mM Tris·Cl, pH 7.5/1 mM EDTA/0.5 M NaCl) containing a protease inhibitor mixture (4). After incubation on ice for 10 min, SDS loading buffer was added, and the cells were boiled for 10 min. Approximately 15 μg of protein was resolved on an SDS/PAGE gel, and the proteins were transferred to nitrocellulose as described (4). The membrane was incubated in blocking buffer [5% Blotto (50 mM Tris·HCl, pH 7.4/100 mM NaCl/5% nonfat dry milk)/0.1% Tween 20] overnight at 4°C. Next, the membrane was incubated with the primary HA antibody (Boehringer Mannheim) at a 1:1,000 dilution for 2 hr at room temperature. After washing the membrane three times in PBS, pH 7.5/0.1% Tween 20, it was incubated with secondary antibody, which was a sheep anti-mouse horseradish peroxidase conjugate (Amersham), at a dilution of 1:4,000 for 1 hr at room temperature. After washing the membrane as described above, the bands were visualized with an enhanced chemiluminescence kit according to the manufacturer's directions (Amersham).

Assay for λ*cII* Mutations. The LN12 cells carrying either β-WT or Y265C were grown to confluence and treated with 25 nM Dex for 48 hr. Genomic DNA was isolated, and the bacteriophage λ shuttle vector was recovered from the genomic DNA extract with a λ *in vitro* packaging extract as described (17). λ*cII* mutants were selected from the packaged genomic DNA by the method of Jakubczak *et al.* (18). The mutation frequency was calculated as the total plaques on the plates with the G1225 lawn divided by the total number of plaques arising on the G1217 lawn.

DNA Sequencing and Mutation Spectra Analysis. The *cII* mutant plaques were picked at random into 100 μl of 10% Tween 20, boiled for 10 min, and then placed immediately on ice for 10 min. Phage DNA was amplified as described by Jakubczak *et al.* (18), purified by using a PCR purification kit (Qiagen), according to the manufacturer's directions, and sequenced by the Keck Center for Biotechnology (Yale University School of Medicine) using the primer 5′-ACC ACA CCT ATG GTG TAT GCA.

RESULTS

To determine whether expression of the Y265C protein in mammalian cells results in a mutator phenotype and to identify the types of mutations that result from overexpression of Y265C, we subcloned the Y265C and β-WT cDNAs into the steroid-inducible pGRE5–2 vector (15) and transfected the resulting constructs into mouse LN12 cells. After transfection, we isolated individual clones by limiting dilution and tested isolates of each transfection for expression of pol β both in the absence and presence of Dex. Because each of the constructs was engineered to include a nucleotide sequence encoding the HA epitope, we employed an antibody that was raised to the HA epitope in Western blot analysis. By using this antibody, we were able to detect expression of pol β from our constructs, rather than expression of the endogenous pol β protein. As shown in Fig. 1, cells that were not induced with Dex to produce either the β-WT or the Y265C protein produced very small quantities of these proteins. However, upon induction with Dex, cells carrying either the β-WT or Y265C cDNA produce more pol βWT-HA or Y265C-HA fusion proteins than when they are not treated with Dex, as shown in Fig. 1. This indicates that expression of the pol β cDNAs from the GRE5 promoter may be leaky, but that pol β expression is induced in the presence of Dex.

Expression of the Y265C Protein in Mouse LN12 Cells Results in an Increase in the Spontaneous Mutation Frequency. To determine whether expression of the Y265C protein results in an increase in the spontaneous mutation frequency, we treated the LN12 cells carrying either the β-WT or the Y265C construct with 25 nM Dex, isolated genomic DNA, packaged the λ shuttle vector, and selected *cII* mutants

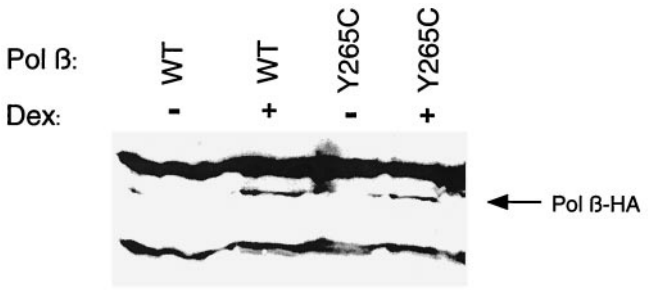

FIG. 1. The β-WT and Y265C proteins are induced in response to treatment with Dex. Approximately 15 μg of protein of each of the different extracts was loaded into each well. The membrane was incubated with a mAb to the HA epitope. The location of the pol β–HA fusion protein is designated with an arrow.

as described in *Materials and Methods*. As shown in Table 1 and Fig. 2, the expression of Y265C in mouse LN12 cells results in an 8-fold increase in the spontaneous mutation frequency compared with β-WT. This demonstrates that mutations arise more frequently in LN12 cells in response to expression of the Y265C protein compared with the β-WT protein.

Expression of Y265C in LN12 Cells Results in Small Deletions. To identify the types of mutations resulting from expression of β-WT or Y265C in the LN12 cells, we determined the sequences of the *cII* mutants. One striking result obtained in our study is that expression of Y265C in LN12 cells results in the production of six deletions of 3 bases or more that were not observed to arise in LN12 cells expressing β-WT protein. The deletion mutations observed when Y265C is expressed in LN12 cells are estimated to arise at a frequency of 1.2×10^{-4}, which is at least 40-fold higher than that observed for β-WT. All but one of the deletions arose in the *cII* sequence between residues 226 and 248, indicating that this sequence is prone to deletion, as shown in Fig. 3. The 3-base deletion between positions 242 and 244 of the *cII* gene was observed three times and most likely occurs as a result of slippage of a GAC repeat. The mechanism of deletion formation of the other three mutants is not readily apparent. These data indicate that expression of the Y265C protein in LN12 cells results in small deletion mutations.

Y265C Produces Different Types of Base-Substitution Mutations than β-WT. As shown in Fig. 2, most of the mutations that resulted from expression of β-WT or the Y265C protein in the LN12 cells were single base-substitution mutations. Expression of Y265C and β-WT resulted in base-substitution mutations arising at a frequency of 6.8×10^{-4} and 0.87×10^{-4}, respectively. Thus, expression of Y265C induces base substitutions at a frequency 7.8 times greater than that of β-WT. To determine whether the base-substitution mutational spectrum we obtained for Y265C was different from that of β-WT, we analyzed our data by using the Adams and Skopek algorithm (19, 20). This analysis yielded a *P* value of 0.000001; because a *P* value of less than 0.05 indicates that we can reject the null hypothesis that the two spectra are the same, our data strongly suggest that the mutational spectrum produced in response to expression of the Y265C protein is different from that produced by β-WT.

Table 1. Mutation frequency of LN12 cells expressing Y265C and β-WT proteins

	cII mutation frequency $\times 10^{-4} \pm$ SE		
Dex	Y265C	β-WT	Y265C/β-WT
+	8.6 ± 2.5	1.0 ± 0.4	8.6
−	2.0 ± 0.4	2.0 ± 0.81	1

Experiments were performed three times. At least 100,000 plaques were scored for Y265C and β-WT in the absence of Dex induction. For Y265C, 746,000 plaques were scored after induction with Dex. For β-WT, 281,000 plaques were scored after induction with Dex. The baseline-mutation frequency for LN12 cells in the absence of either Y265C or β-WT expression systems is $2.4 \pm 0.3 \times 10^{-4}$.

Expression of the Y265C mutant in LN12 cells produced predominantly GC-to-AT transitions at a frequency of 47×10^{-5}, which is 15-times greater than those resulting from expression of β-WT protein, as shown in Table 2. In contrast, predominantly three types of mutations, GC-to-AT transitions, GC-to-TA transversions, and AT-to-GC transitions, were produced in cells expressing β-WT, with the latter two types of mutations arising at frequencies nearly equal to that observed for Y265C. These data show that expression of Y265C in LN12 cells results in base-substitution error specificities that are significantly different from those observed with β-WT.

DISCUSSION

In this study we demonstrate that expression of the Y265C protein in mouse LN12 cells results in a mutator phenotype. This result strongly suggests that the Y265C protein is dominant to the endogenous pol β protein and that the mutations that arise in the LN12 cells expressing Y265C result directly or indirectly from the action of this mutator protein.

Our mutational target in the mouse LN12 cells is the *cII* gene of bacteriophage λ. This gene is present on a shuttle vector, 100 copies of which are integrated into the genome of the LN12 cells. To identify the types of mutations produced by Y265C and β-WT, we isolated the *cII* mutants induced by these proteins and determined their DNA sequences. We obtained a significantly different mutational spectrum, as determined by analysis with the Adams and Skopek algorithm, for the Y265C mutant relative to β-WT. Expression of Y265C results in a 40-fold increase in small deletions compared with β-WT. In addition, Y265C induces predominantly GC-to-AT transitions, whereas expression of β-WT results in nearly equal proportions of GC-to-AT, GC-to-TA, and AT-to-GC base-substitution mutations. These data demonstrate that Y265C has a different mutational signature than β-WT when expressed in mouse LN12 cells and indicates that it is dominant to the endogenous pol β protein.

Expression of Y265C Results in an Increased Frequency of Deletion Formation. We obtained at least a 40-fold increase in the frequency of deletions of 3 bases or more in LN12 cells expressing Y265C compared with β-WT. This result suggests that Y265C plays either a direct or an indirect role in the formation of small deletions. As shown in Fig. 3, three of the *cII* mutants had an ACG deletion that was part of a GAC-repeated sequence at positions 239–244. Because this deletion was within a GACGACGAC-repeated sequence, it is likely that it occurred by a slippage-mediated mechanism (21, 22). The mechanism of deletion formation in the other three *cII* mutants is not readily apparent. The breakpoints of the deletion that occurred between positions 230 and 248 are repeats of G residues, indicating that a recombination event may have taken place, resulting in the observed deletion. The other two deletions shown in Fig. 3 do not have repeated sequences at their ends and do not seem to be part of a slippage-mediated event.

It has been shown previously that pol β functions in base excision repair (BER) *in vivo* (3). Presumably, the Y265C and β-WT proteins that we expressed in the mouse LN12 cells also function in BER, during the gap-filling step. Because the growth of the cells does not seem to be altered upon expression of these proteins (C.C. and J.B.S., unpublished data), we do not think that they are acting during DNA replication; if they did function in DNA replication, we would expect that the Y265C dominant-negative protein would inhibit this process,

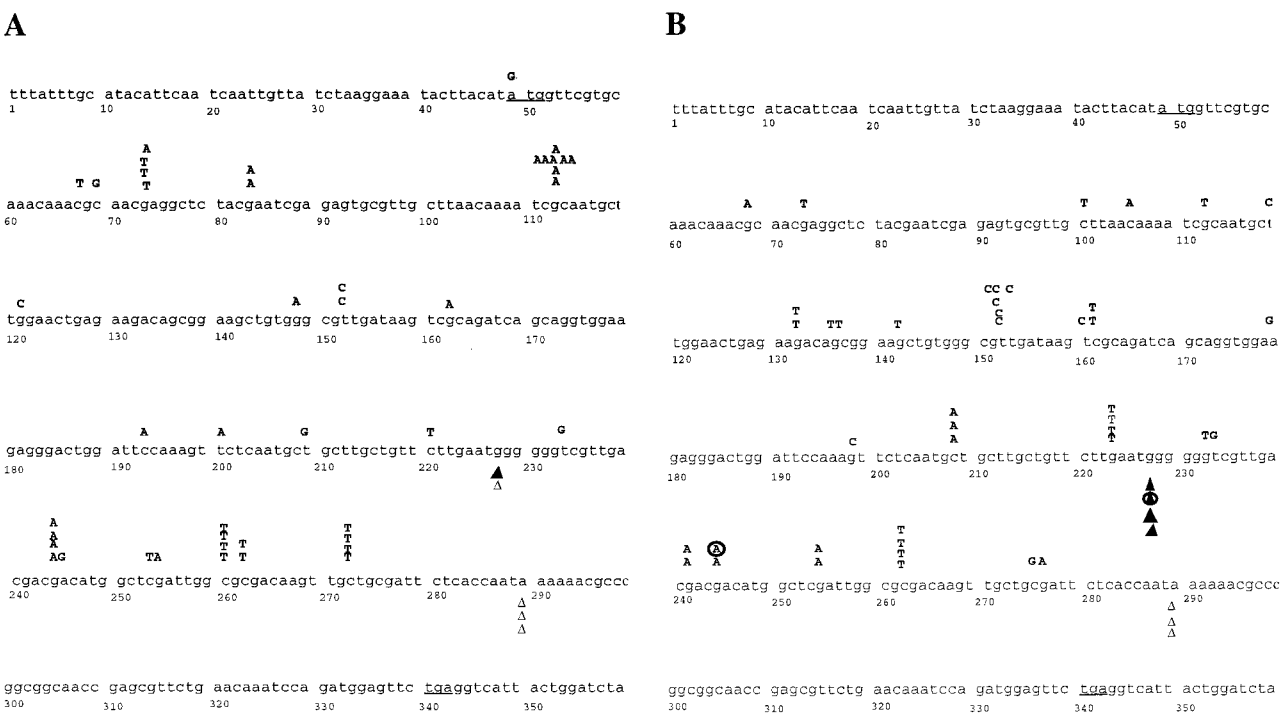

FIG. 2. Mutation spectra of Y265C and β-WT. (*A*) β-WT. (*B*) Y265C. The sequence of the λ *cII* gene is shown. All base-substitution mutations and one-base-pair frameshift mutations are shown in the spectra. Base substitutions are above the *cII* sequence; single base deletions (△) and single base insertions (▲) are below the sequence at the site at which they were observed. The circled G → A and single base-insertion mutations were present in one *cII* mutant. These mutations are considered as separate events because they are greater than 15 bases apart (13). Not included in the Y265C spectrum are the deletions occurring between 140–154, 242–244, 226–236, and 230–248; these are shown in Fig. 3. Not included in the β-WT spectrum is the deletion of AA at position 60–61.

resulting in a decreased doubling time of the cells. In addition, preliminary experiments indicate that expression of Y265C slightly sensitizes the LN12 cells to alkylating agents, suggesting that it is acting during BER. However, we cannot completely rule out the possibility that these proteins are functioning in other cellular processes.

Pol β functions predominantly in filling 1-base gaps during BER, although it seems to be able to participate in long-patch BER as well (23–25). Therefore, the formation of the observed deletions most likely occurred during either short- or long-patch BER. The deletion of the ACG repeat at positions 242–244 may have occurred during the filling of a 6-bp gap by pol β. However, it is unlikely that very large gaps form during BER (26); therefore, the formation of the other deletions probably did not occur simply during the filling of a large gap. One possibility is that Y265C binds to a gap in the DNA

cII Sequence:

5'-gcttgctgttcttgaatgggggtcgttgacgacgacatggctcgattggcgcgac

Deletions:

242-244(3)[1] 5'-gcttgctgttcttgaatgggggtcgttgacg...acatggctcgattggcgcgac

230-248(1) 5'-gcttgctgttcttgaatgg.....ggctcgattggcgcgac

226-236(1) 5'-gcttgctgttcttgaa.....tgacgacgacatggctcgattggcgcgac

cII Sequence:

5'-aagacagcggaagctgtgggcgttgataagtcgcag

Deletion:

140-154(1) 5'-aagacagcgg.....ataagtcgcag

FIG. 3. Deletion mutations identified in the LN12 cells expressing the Y265C protein. Six deletions greater than 2 bases were identified in *cII* mutants arising from cells expressing the Y265H mutant; no deletions of this type were observed in *cII* mutants arising from cells expressing the β-WT protein. [1]The numbers indicate the positions within the *cII* gene, and the number in parentheses is the number of times the deletion was observed.

Table 2. Base-substitution error specificity of Y265C and β-WT in the λ*cII* assay

Base-substitution class	Mispair*	Frequency × 10^{-5} (no. observed)		Y265C/ β-WT†
		Y265C	β-WT	
GC → AT	G:T or C:A	47 (31)‡	3.1 (15)§	15
GC → TA	C:T or G:A	6.2 (4)¶	2.9 (14)‖	2.1
AT → GC	A:C or T:G	6.2 (4)	1.8 (9)	3.4
GC → CG	G:G or C:C	3.0 (2)	0.4 (2)	7.5
TA → GC	T:C or A:G	3.0 (2)	0.2 (1)	15
TA → AT	T:T or A:A	1.5 (1)	0.2 (1)	7.5
Total**		44	42	

*A mispair that could be formed by the Y265C or β-WT polymerase.
†The frequency of mutations arising from expression of Y265C divided by the frequency of mutations arising from expression of β-WT.
‡Twenty-three of 31 transitions are at CpG dinucleotides.
§Twelve of 15 transitions are at CpG dinucleotides.
¶Three of 4 transversions are at CpG sites.
‖Two of 14 transversions are at CpG sites.
**Total number of base-substitution mutations.

resulting from excision of an adducted base or a single-strand break. Y265C remains at this gap for a longer amount of time than β-WT, without insertion of a deoxynucleotide. This could result in the formation of a larger gap or double-strand break. The repair of this intermediate might result in a deletion. Evidence providing support for this hypothesis includes our finding that Y265C has a 4-fold-decreased K_m for gapped DNA, is 10 times less active than β-WT protein, and interferes with BER in *S. cerevisiae*, most likely because it binds to small gaps and blocks other *S. cerevisiae* polymerases from gaining access to them (10, 11, 14). Alternatively, the deletions could be formed during another cellular process.

Few One-Base-Pair Frameshift Mutations Result from Expression of β-WT or Y265C. Few one-base-pair frameshift mutations arose in LN12 cells expressing either β-WT or Y265C, as shown in Fig. 2. Approximately 9 and 15% of the mutations produced by Y265C or β-WT, respectively, were one-base-pair frameshifts. This is similar to results presented by Jakubczak *et al.* (18), who also observed that few one-base-pair frameshifts were produced within the *cII* gene *in vivo*. Each of the one-base-pair frameshifts we detected for β-WT and Y265C occurred in either a run of G residues between positions 227 and 232 or at a run of A residues between positions 289 and 294 of the *cII* gene, suggesting that a slippage-mediated event contributed to the formation of the one-base-pair frameshift mutations.

Y265C Produces Predominantly GC-to-AT Transitions. The largest category of mutations we observed were base-substitution mutations. In the mouse LN12 cells expressing Y265C, approximately 31 of 44 of the base substitutions we detected were GC-to-AT transitions, 80% of which were located at CpG sites. In contrast, expression of β-WT resulted in only 15 of 42 of the base substitutions being GC-to-AT transitions, 80% of which were at CpG dinucleotides; 14 of 42 of the mutations induced by β-WT were GC-to-TA transversions, and 9 of 42 were AT-to-GC transitions.

One explanation for the observation that mutations arise in different proportions in cells expressing Y265C compared with β-WT is that Y265C possesses an intrinsic error specificity that is different from that of β-WT. If Y265C is functioning in BER, it could commit errors during the gap-filling step that result in mutations. The predominant type of mutation induced in cells expressing Y265C is a GC-to-AT transition at a CpG dinucleotide, which is a hotspot for deamination. Deamination of 5-methylcytosine results in thymine and a G:T mispair. A thymine DNA glycosylase can recognize and excise the T from the G:T mispair, leaving a small gap, which is filled in by Y265C. If Y265C misinserts T opposite G, a GC-to-AT transition will result. Alternatively, this mispair can be replicated, giving rise to a GC-to-AT transition. We postulate that Y265C is playing a direct role in the mutational process by misinserting T opposite template G during BER mainly because when Y265C copies the HSV-*tk* gene *in vitro*, the most frequent mispair that is formed is G:dTMP (13). For GC-to-AT transitions that do not arise at CpG sequences, a G lesion, such as 8-oxoG, might initiate BER. For the transition to be observed, Y265C must insert A opposite template C. Formation of a C:dAMP mispair is the second most frequent mispair formed *in vitro* by Y265C. Expression of Y265C in the LN12 cells also results in GC-to-TA transversions and AT-to-GC transitions at frequencies nearly equal to β-WT, implying that the mispair formed by Y265C that will result in these mutations is not one that this enzyme forms often. The GC-to-TA transversions arise at CpG dinucleotides, indicating that Y265C must misinsert A opposite template G for the mutation to be observed. When copying the HSV-*tk* gene *in vitro*, Y265C rarely formed the G:dAMP mispair. The AT-to-GC transitions could result from misincorporation of C opposite template A or G opposite template T, both of which are rarely formed *in vitro*. Thus, the mutational signature of Y265C *in vivo* and *in vitro* is quite similar.

An alternative explanation for the possibility that Y265C, but not β-WT, has the same mutational signature both *in vivo* and *in vitro* is that Y265C appears to be dominant to β-WT in *E coli* and to other DNA polymerases in *S. cerevisia*e. Thus, during BER in the LN12 cells, Y265C would compete successfully with β-WT or another endogenous polymerase for the gapped DNA substrate. It could fill in gaps that would most likely be filled in by polymerases other than pol β in addition to gaps that are likely to be filled in by pol β. During gap filling, Y265C could commit an error resulting in a mutation. Thus, the mutations it induces *in vivo* and *in vitro* would be similar. However, when β-WT is expressed in the LN12 cells, it most likely does not compete with other DNA polymerases for gapped DNA as strongly as the Y265C variant and fills in gaps that most likely would be filled in by the endogenous pol β. The mutational spectrum we obtain arises from all mutational events in cells, including gap filling by pol β and other DNA polymerases. Thus, the mutational pattern we observe for β-WT in LN12 cells actually might result from errors committed by pol β and other DNA polymerases during gap filling and would not be expected to bear the *in vitro* mutational signature of wild-type pol β.

In summary, we have shown that expression of Y265C confers a mutator phenotype to mouse LN12 cells. Our data suggest that the action of Y265C during BER is directly responsible for the mutations we identified. The generation of a mutator phenotype in cells has been postulated to have a role in the onset and progression of neoplastic disease (27–29). Our data indicate that alterations within the pol β gene have the potential to result in a mutator phenotype in mammalian cells, especially if the alterations result in a protein that is dominant to the wild-type pol β enzyme.

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