[CANCER RESEARCH 62, 3251–3256, June 1, 2002]

Splice Variants but not Mutations of DNA Polymerase β Are Common in Bladder Cancer

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ABSTRACT

DNA polymerase β (POLβ) is a highly conserved protein that functions in base excision repair. Loss of the *POLβ* locus on chromosome 8p is a frequent event in bladder cancer, and loss of POLβ function could hinder DNA repair leading to a mutator phenotype. Both point mutations and large intragenic deletions of *POLβ* have been reported from analysis of various tumor cDNAs but not from genomic DNA. We noticed that the breakpoints of the presumed rearrangements were delineated by exon-exon junctions, which could instead be consistent with alternative splicing of *POLβ* mRNA. We tested the hypothesis that the reported intragenic deletion were splice variants by screening genomic DNA of human bladder tumors, bladder cancer cell lines, and normal bladder tissues for mutations or deletions in exons 1–14, exon α, and the promoter region of *POLβ*. We found no evidence of somatic mutations or deletions in our sample set, although two polymorphisms were identified. Examination of cDNA from a subset of the original sample set revealed that truncated forms of *POLβ* were surprisingly common. Forty-eight of 89 (54%) sequenced cDNA clones had large deletions, each beginning and/or ending exactly at exon-exon junctions. Because these deletions occur at exon-exon junctions and are seen in cDNA but not genomic DNA, they are consistent with alternative mRNA splicing. We describe 12 different splicing events occurring in 18 different combinations. Loss of exon 2 was the most frequent, being found in 42 of 49 (86%) of the variant sequenced clones. The splice variants appear to be somewhat more common and variable in bladder cancer cell lines and tumor tissues but occur at a high frequency in normal bladder tissues as well. We examine alternative splicing in terms of the information content of splice donor and acceptor site sequences, and discuss possible explanations for the predominant splicing event, the loss of exon 2.

INTRODUCTION

The POLβ[2] protein performs a critical role in base excision repair of DNA. Its primary role is gap-filling DNA synthesis, although it appears to be a required cofactor in multiple steps both before and after DNA synthesis. The loss of POLβ activity in a cell impairs base excision repair capacity and can lead to the development of a mutator phenotype (1). Such a phenotype might increase the frequency of mutations in tumor suppressor genes, proto-oncogenes, or other important growth control genes, and increase the probability of cancer.

POLβ is a single-copy gene mapped to chromosome 8p11-p12 (2). Deletions of 8p are a relatively frequent event in cancer having been shown in prostate (3), colorectal (4), stomach (5), breast (6), lung (7), and kidney tumors (8), and are particularly common in bladder cancers (25%; Ref. 9). Losses in this area are associated with more aggressive colorectal, hepatocellular, breast, and bladder cancers, and may be an early event in breast cancers (7, 10–12).

Various mutations of *POLβ*, including large deletions, insertions, and some point mutations, have been reported in studies using cDNA from colorectal, bladder, and prostate tumors (13–15). Although originally reported as mutations, several of the deletions fall at exon-exon junctions and would be consistent with splice variants. Such deletions have been demonstrated in cDNA (16), but genomic DNA has not been sequenced from these samples. Large deletions and insertions that do not occur at known exon junctions may represent true somatic mutations, although they have yet to be demonstrated in genomic DNA.

We have screened genomic DNA from human bladder cancer cell lines along with primary human bladder for mutations in *POLβ*. Splice variants were distinguished from genomic mutations or inherited polymorphisms by comparing cDNA and genomic sequences from matched tumor and normal tissue from bladder cancer patients. Neither point mutations nor deletions were found in genomic DNA; however, two polymorphisms and extensive alternative splicing of *POLβ* was detected in cell lines, normal, and tumor samples.

MATERIALS AND METHODS

Cell Lines, Clinical Samples, DNA, and RNA Isolation

Transitional cell carcinomas and matching normal tissues were collected at University of North Carolina Hospitals, Roswell Park Cancer Institute, and Duke University Medical Center as described previously (17). Bladder tumors were flash frozen, cut in 10-μm sections, and mounted on slides. Bladder cell lines were obtained from American Type Culture Collection (Rockville, MD) and maintained in DMEM/F12 with 100 units of penicillin, 100 mg/ml streptomycin, and 10% fetal bovine serum in a 5% carbon dioxide humidified incubator. DNA from cell lines, fresh-frozen bladder tumors, and normal bladder tissue was extracted using standard methods (18). Total RNA from selected cell lines, fresh-frozen bladder tumors, and paired, fresh-frozen normal tissue were extracted using standard methods (18).

Genomic DNA Analysis

PCR. The 14 exons and exon α of *POLβ* (19) were amplified from genomic DNA using primer sequences located in the introns flanking each exon (Table 1). The complete 28 nucleotide acceptor sites and 10 nucleotide donor sites used in splicing were included in the amplification fragment at each exon (20). The promoter region was amplified using a primer located in exon 1 and in the 5′ flanking intronic region that encompasses the entire putative promoter region (21).

DNA (25 ng) was added to a master mix containing 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 200 μM dGTP, dCTP, dTTP, and dATP; 0.75 mM of each primer; 1 unit of Taq polymerase (PE Biosystems, Foster City, CA); and between 0.8 mM and 3.0 mM $MgCl_2$. Amplifications were performed for 35 cycles in a Perkin-Elmer 9700 thermal cycler using standard conditions at the empirically determined annealing temperatures (Table 1).

SSCP Analysis. PCR amplifications for SSCP were done as reported above except with 1 μCi of [α-^{33}P]dATP (Amersham Corp., Arlington Heights, IL) and only 20 mM dATP. PCR product (4 μl) was denatured at 95°C for 5 min in 40 μl of SSCP denaturing loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.02% xylene cyanol FF, and 0.02% bromphenol blue) and loaded on a ×0.5 Mutation Detection Enhancing gel (AT Biochem, Malvern, PA), and

Received 8/23/00; accepted 3/28/02.

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[2] The abbreviations used are: POLβ, DNA polymerase β; SSCP, single-strand conformational polymorphism; UTR, untranslated region.

Table 1 *Primers used for PCR amplification of exons 1 through 14, exon α, the putative promoter region, and full-length cDNA*

Exon	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Annealing temperature	Magnesium +2 concentration
1 and 2	TCG CGC CGG AGC TGG GTT GTT	GGC TGG AAG GAA AGA AGA AAG	60	1.0
3	GGC CTT GAT GGA TTT CTA AT	TTT GAA CAC GTG TGA ACT CT	50	1.5
4	TCT GCT TTT TAC TTT ATC TT	CAC CCT GCA ATG ACG CTA CA	50	3.0
5	CTC TTC ATG TCT TTT AGC AG	GTG GTG AAG TAC AGG TTT TG	50	1.0
6	TTT AGC AGG TCT TGT TTA GC	CTG ATT GAG AAT GGG TTT TT	50	2.0
7	GGG TCA TTG AGT TTA GCA TT	GAA GAG TCC TTC TGG TGT AT	50	3.0
8 and 9	ATA GAT ATT TTG GGG ACT TTG	TTA GGC AAT TTT AAC ATC AGG	50	1.0
10	TTG CCC AAT TGA TAC ATT TAC A	GGA AAC AAA ACT TAT TCT GAA A	50	1.5
11	AAT TAC TCT TTT TCT TAT TCC	GAT GTG CTA ACT CTA ACA AGT	50	1.5
12 and 13	TGG CCT TGT GTT TTA CTT GAT	GGC TCT AGA TAT GAA TGT GAA	50	1.5
14	TGA AAG CAA GTC CCA CAC AGC	CCA AAG ACC CTT ACA TAG CAA	55	1.5
α	GCT TAT CTT GCT GTT TCT TTA	GAT TGC GCC ACT GTA TTT CTG	55	1.0
Promoter	CCA CGA GTC CAC GAA CCC TCC	CTC CTG CGG CGC CTT CC	70	1.5
cDNA	TGC TCC TGC TCC CGT CTC CAA	CCA AAG ACC CTT ACA TAG CA	55	1.5

electrophoresed in 0.6 × Tris-borate-EDTA buffer at 7 W for 16 h at room temperature. After electrophoresis, the gel was dried and exposed to autoradiographic film (Eastman Kodak, Rochester, NY) overnight at room temperature.

Manual Sequencing of Samples Displaying Shifts. Samples displaying variant bands on SSCP were additionally analyzed by direct sequencing. DNA samples were amplified by PCR as described above. The amplification products were purified with a Wizard PCR Prep kit (Promega, Madison, WI).

Purified PCR product (4 μl) was sequenced using Thermo Sequenase radiolabeled terminator kit according to protocols provided by the manufacturer (Amersham). The sequencing products were denatured and electrophoresed on 6% polyacrylamide gels containing 8.3 M urea. The gels were fixed in 10% methanol/10% glacial acetic acid, dried, and exposed to autoradiographic film (Eastman Kodak) overnight at room temperature.

cDNA Analysis

Reverse Transcription-PCR. Reverse Transcription-PCR was carried out for samples from three patients where there was adequate matched tumor and normal bladder tissue and for two cell lines. Total RNA (5 μg) was allowed to denature and anneal to random hexamers (New England BioLabs, Beverly, MA) in the presence of RNasin at 70°C. The reverse transcription reaction, using Superscript II RNase H minus Reverse Transcriptase (Life Technologies, Inc., Rockville, MD), proceeded at 37°C for 15 min and then 45°C for 1 h.

PCR Amplification of *POLβ* cDNA. Full-length cDNA amplifications were done using Expand High Fidelity PCR System (Roche Diagnostics Corporation, Indianapolis, IN) following manufacturer's recommendations. Primers for PCR amplification of cDNA (Table 1) were located in the 5′ and 3′ UTRs of *POLβ* exons 1 and 14, respectively, producing a product of 1189 bp including the entire coding region.

Southern Blotting. *POLβ* cDNA was PCR amplified as described above from total cDNA for the matched tumor and normal sets and the two bladder cancer cell lines. *POLβ* cDNA was also PCR amplified directly from four selected cDNA clones (see below) isolated from cell line HT 1376. These amplified cDNAs were electrophoresed on a 6% nondenaturing acrylamide gel at 150 V for 4 h. The samples were then transferred onto nylon filter paper by electrophoresis (22). The filter was denatured and neutralized as described below. The filter was allowed to dry and was UV-linked. The filter was equilibrated with Hybrisol I containing 100 μg/ml Salmon Sperm DNA at 42°C for 1 h. A probe radiolabelled with ^{33}P and made from full-length *POLβ* was hybridized to the filter. The filter was then washed and exposed overnight to autoradiographic film at room temperature.

Ligation and Transformation. PCR-amplified *POLβ* cDNA from the three matched bladder tumor and normal sample sets and from two bladder cancer cell lines was purified using a Wizard PCR purification kit (Promega) and ligated into either the pcr2.1 or the PCRscript vector (Invitrogen, San Diego, CA; Stratagene, La Jolla, CA) according to manufacturer protocols. After ligation, vectors were transformed into XLblue *Escherichia coli* following standard protocols. Cells were plated at various densities onto agar containing 2% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, 0.1 mM isopropyl-1-thio-β-D-galactopyranoside, and 25 μg/ml ampicillin then incubated at 37°C, inverted, for 20 h.

Probing Clones for Insert. Clones containing insert lose α-complementation for β-D-galactosidase and are white, whereas clones without insert are blue. White clones were randomly picked and streaked onto parallel plates. To exclude clones containing T-tailed insert without *POLβ* sequence, we screened clones by Southern analysis: one of the parallel plates was blotted with filter paper, the filters were lysed, denatured, neutralized, and allowed to dry. Afterward, they were baked in vacuum at 80°C for 15 min, equilibrated, and hybridized as described for Southern blotting with a radiolabelled full-length *POLβ* probe. The filters were washed and exposed to autoradiographic film. Any clone showing a *POLβ* insert was located on its parallel plate, inoculated into Luria-Bertani broth containing 25 μg/ml ampicillin, and grown shaking at 37°C overnight. The culture was pelleted and the plasmid purified from the cells by use of the Wizard Miniprep purification kit (Promega) and used for automated sequencing.

Automated Sequencing of Plasmids. Plasmids containing *POLβ* cDNA were sequenced using a Model 377 Automated Sequencer (ABI; Perkin-Elmer, Foster City, CA). Purified plasmid DNA was used as template in a reaction including sequencing primers and Rhodamine Fluorescence Ready Reaction sequencing kit (Perkin-Elmer). Sequencing primers were specific for the vector being used to amplify the region cloned into the vector.

Splice Site Information Analysis

Although mammalian splice sites exhibit considerable sequence variability, they can be accurately and comprehensively evaluated in genomic sequences with computational approaches based on information theory (23). Briefly, the information content of 28-nucleotide acceptor or 10-nucleotide donor sequence used as a binding site by proteins of the spliceosome can be quantified in bits as R_i. The mean R_i is the average amount of information required for splicing. For acceptor sites R_i averages 9.35 ± 0.12 bits and for donor sites averages 7.92 ± 0.09 bits (20). Strong splice sites have values exceeding the mean, whereas weak splice sites have values much less than the mean. Nonfunctional sites have $R_i \leq 0$.

Alternative spliced acceptor sites were evaluated by comparing the R_i values (Table 2) of the upstream sites of exons that were excluded with downstream sites that were used for the alternatively spliced cDNA clones.

Table 2 *R_i values for each exon of DNA polymerase β*

Exon	Acceptor R_i value (bits)	Donor R_i value (bits)
1	—	6.8
2	11.1	8.0
3	15.6	8.5
4	7.6	6.9
5	11.5	7.3
6	6.7	12.2
7	10.9	6.4
8	11.4	7.6
9	9.7	5.5
10	11.5	5.1
11	6.6	10.5
12	3.0	4.0
13	10.6	10.6
14	12.8	—

Relative strengths of alternatively spliced pairs of acceptor sites were measured by computing the minimum fold difference in binding affinity, $2^{\Delta R_i}$ where ΔR_i is the difference between the R_i value of the natural site and that of the variant site (24–26).

RESULTS

Genomic DNA Analysis. Genomic DNA from 11 bladder tumors and 8 bladder cancer cell lines was screened by SSCP for mobility shifts in exons 1–14, exon α, and the core promoter region of *POLβ*. A bandshift was observed when screening exon 14 in 3 of 11 bladder tumors (Fig. 1) and 0 of 8 cell lines. Because of the frequency, this change was suspected to be a polymorphism. Direct sequencing revealed it to be, in all of the cases, a thymidine inserted into a string of five thymidines 17-bp upstream of the intron 13/exon 14 junction. This extra thymidine would increase the length of the pyrimidine run in the acceptor site of exon 14. Calculation of R_I, and the information content (23) of both the wild-type and variant versions of the acceptor sites show this insertion increases the information content of the acceptor site from 12.8 bits to 13.2 bits. This difference corresponds to a 1.3-fold increase in the strength of the spliceosome-splice site interaction and would be predicted to make exon 14 somewhat more likely to be spliced into a finished transcript in samples containing the polymorphism.

One of 8 bladder cancer cell lines and none of the 20 bladder tumor samples showed a bandshift in exon 12. Direct sequencing showed this to be a CCC → CGC transversion at codon 242 that results in a proline to arginine amino acid change and has been reported previously as a polymorphism (27). No other abnormalities were detected by SSCP in any samples in exons 1–14, exon α, or the promoter region of the gene.

cDNA Analysis. We examined cDNA made from tumor tissue and from normal bladder tissue for three bladder cancer patients and from two bladder cancer cell lines. Primers used to PCR amplify *POLβ* cDNA from total cDNA were located in the 5′ UTR at the beginning of exon 1 and in the 3′ UTR after the end of exon 14, so that only transcripts that include these regions are available for analysis. PCR-amplified *POLβ* cDNA from the three match tumor and normal bladder samples, and from both bladder tumor cell lines when hybridized against a full-length *POLβ* probe, revealed multiple bands on Southern blotting (Fig. 2, *Lanes 1–8*).

Cloning and sequencing of 89 *POLβ* cDNAs from these samples confirm a wide variety of *POLβ* cDNAs in all of the specimens (Fig. 3). Full-length *POLβ* cDNA, which we define as exons 1–14 but excluding exon α, was seen as the most prevalent species in all eight of the samples, being identified in 40 of 89 (45%) of the *POLβ* cDNA-containing clones that were sequenced (see Fig. 3). Variant *POLβ* cDNA was found in 49 of 89 (55%) of the sequenced clones

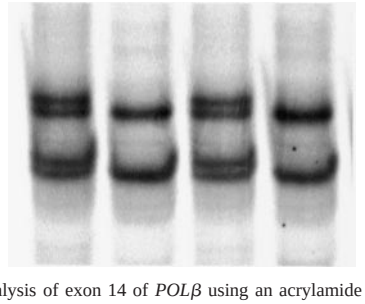


Fig. 1. SSCP analysis of exon 14 of *POLβ* using an acrylamide gel. Band shifts are visible in *Lanes 1* and *3*. Shifts were observed in 3 of 11 bladder tumor samples (27%) and 0 of 8 bladder cancer cell lines. Direct sequencing revealed this shift to be, in all cases, a thymidine inserted into a string of five thymidines 17-bp upstream of the intron 13/exon 14 junction.

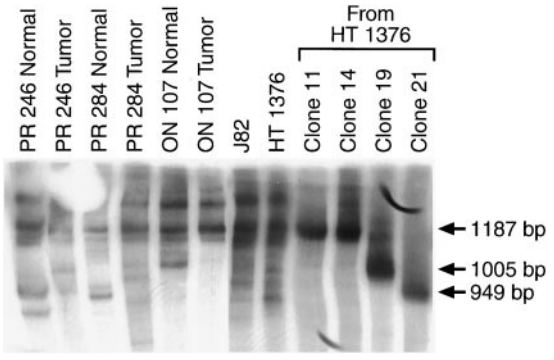


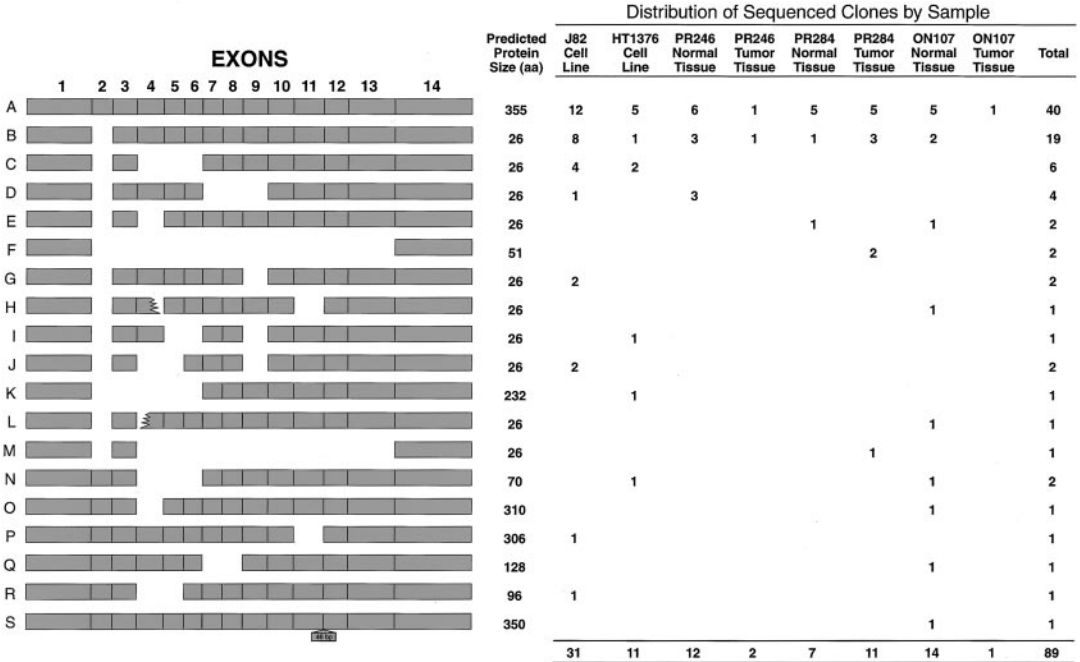
Fig. 2. Southern blot of amplified cDNA using full length *POLβ* as probe. *Lanes 1–8* PCR amplified *POLβ* from tissue sample total cDNA demonstrating the presence of multiple size variants. Size variants are present in both tumor and matched normal bladder epithelium. A subset of the size variants for each sample were subsequently cloned and sequenced (see Fig. 3). *Lanes 9–12* PCR amplified *POLβ* from a selected subset of 4 of the 11 fully sequenced clones derived from bladder cancer cell line HT1376 including two full-length clones (clones 11 and 14, 1189 bp), one clone missing exons 4 through 6 (clone 19, 1005 bp), and one clone missing exons 2, 5, 6, and 9 (clone 21, 949 bp).

from cell lines, tumor, and normal tissues, and represents 18 variant splicing species (Fig. 3). Four of the 11 sequenced cDNA clones from cell line HT1376 representing two full-length and two truncated *POLβ* cDNAs were PCR amplified and included in Southern blot analysis (Fig. 2, *Lanes 9–12*), and demonstrate that the cloned and sequenced bands correspond in size to bands visible in amplified cDNA from the cell line (Fig. 2, *Lane 8*). Whereas the 18 variants identified from sequencing 89 clones has captured a large portion of the variant *POLβ* cDNAs present in these samples, there are additional variants present in these samples that have not been cloned and sequenced. This includes a band larger than the full-length *POLβ* cDNA that was evident in the Southern analysis of most samples and that remains unidentified.

Sequencing of clones demonstrated that exons 2, 4, 5, 6, and 9 were the most often omitted from cDNA and were found omitted alone or in combination with other exons. Forty-eight of the 89 sequenced clones (54%) were truncated cDNA. All but 2 of these variants had omissions of entire exons with the deleted region beginning and ending exactly at exon-exon junctions. Two variants (variants H and L, Fig. 3) had portions of exons omitted in addition to missing exon 2. Both partial deletions, while not entirely encompassing an entire exon, began or ended exactly at the exon-exon junction. A single clone (variant S, Fig. 3) displayed a 46-bp insert added exactly at the junction between exons 11 and 12. This clone was sequenced in two directions to confirm the 46-bp insert of GGAAGAGGAA GCACTC-GAGG TCAACTTTCA AATCAATTTT AGAGAC. The insert was not part of the published sequence of intron 11 nor did it contain sequence from exon α, which has been reported as an insertion between exons 7 and 8 (19).

POLβ cDNA missing only exon 2 was the most prevalent splice variant and was observed in 19 of 89 sequenced clones (24%), and has been reported by several other groups (27, 28) as both a deletion and a splice variant. Often exon 2 was lost in combination with other exons (23 of 89 clones). Of the 18 species of variants observed, 12 were missing exon 2. Overall, exon 2 was absent, alone or in combination with other exons, in 47% (42 of 89) of all of the sequenced clones and 86% (42 of 49) of the variant clones.

Interestingly, loss of exon 2 was most often followed by inclusion of exon 3 even when subsequent exons were lost. Of the 12 species of variants missing exon 2 (Fig. 3, *B-M*) 10 had an intact exon 3. When this occurs, an early stop codon is created in exon 3 that would be predicted to produce a truncated protein of only 26 amino acids. In the



	Predicted Protein Size (aa)	Distribution of Sequenced Clones by Sample: J82 Cell Line	HT1376 Cell Line	PR246 Normal Tissue	PR246 Tumor Tissue	PR284 Normal Tissue	PR284 Tumor Tissue	ON107 Normal Tissue	ON107 Tumor Tissue	Total
A	355	12	5	6	1	5	5	5	1	40
B	26	8	1	3	1	1	3	2		19
C	26	4	2							6
D	26	1		3						4
E	26					1		1		2
F	51						2			2
G	26	2								2
H	26							1		1
I	26		1							1
J	26	2								2
K	232		1							1
L	26							1		1
M	26						1			1
N	70		1					1		2
O	310							1		1
P	306	1								1
Q	128							1		1
R	96	1								1
S	350							1		1
		31	11	12	2	7	11	14	1	89

Fig. 3. Summary of results obtained from sequencing of 89 *POLβ* cDNA clones derived from tumor and normal bladder tissue and from bladder cancer cell lines. Full length *POLβ* (row A) plus 18 different species of splice variants (rows B-S) are identified Skipped exons are shown in white. The number of sequenced clones from different tissues and cell lines are presented in tabular form. Exon 2 was the most frequently skipped exon and would be predicted to produce a truncated protein of 26 amino acids caused by a stop in exon 3.

2 variants where loss of exon 2 was also followed by loss of exon 3, the predicted protein would be longer, 232 or 51 amino acids (Fig. 3).

Splice Site Information. Table 2 shows the R_i values of the acceptor and donor sites of each exon. All of the acceptor sites had R_i values in the normal range (20). We investigated the possibility that differences in information contained in acceptor sites could account for the presence of the splice variants observed. Experimental studies have shown that the acceptor sites of alternatively spliced exons exhibit weaker splicing signals or contain suboptimal polypyrimidine tracts compared with constitutively spliced exons (29). To address this hypothesis for *POLβ*, the relative strengths of alternatively spliced and skipped acceptor sites were compared (Table 3).

Twelve different varieties of single or adjacent exon skipping were observed from the 89 sequenced clones and are listed in Table 3. Each represents a specific alternative splicing event. The 17 alternative splicing species identified in Fig. 3 (*B-R*) are formed from different combinations of these 12 varieties of alternative splicing events.

In 7 of 12 of the specific alternative splicing events, representing 41 of 49 alternatively spliced clones, the R_i value of the acceptor site that was used exceeds the corresponding value for the upstream site that would have been used had splicing been constitutive (Table 3). Differences between the strengths of these pairs of sites ranged from 1.3- to 37-fold, with the most abundant variant (lacking exon 2) exhibiting 23-fold difference between the alternative and constitutive splice sites. In instances where multiple exons are skipped, the internal acceptor sites were in some instances stronger than the site that was used, consistent with the possibility that splice site recognition may not be processive (30). The R_i values of the downstream acceptor sites did not exceed those of the corresponding upstream sites in 5 of 12 alternative splicing events (Table 3) but represented only 8 of 49 alternatively spliced clones.

DISCUSSION

Loss of heterozygosity of chromosome 8p, including the *POLβ* locus, is a frequent event in bladder tumors. Loss or mutation leading to the inactivation of *POLβ* can give rise to a chromosome instability

Table 3 *Relative strengths of used exon acceptor sites over skipped exon acceptor sites in alternatively spliced exons*

Exon(s) skipped	Splice variant[a]	Exon acceptor used, skipped[b]	Change in R_i (bits)[c]	Minimum fold, binding difference[d]	Normal tissue, number of clones[a]	Cell lines and tumors, number of clones[a]
2	B, C, D, E, G, H, I, J, L, M	3, 2	5.4	23	13	28
2 to 13	F	14, 2	1.7	3.2	0	2
4	E, O	5, 4	3.9	15	2	0
4 to 6	C, N	7, 4	3.3	9.8	1	6
4 to 13	M	14, 4	5.2	37	0	1
7 to 9	D	10, 7	0.6	1.5	3	1
9	G, I, J	10, 9	1.8	3.5	0	5
2 to 6	K	7, 2	−0.2	0.9	0	1
4 to 5	J, R	6, 4	−0.9	0.5	0	3
5 to 6	I	7, 5	−0.6	0.7	0	1
7 to 8	Q	9, 7	−1.2	0.4	1	0
11	H, P	12, 11	−3.6	0.08	1	1

[a] See Fig. 3 for map of splice variants and clones. Variants and clones with multiple splicing are represented more than once since they contribute more than one splicing event.
[b] Exon number of used acceptor site and exon number of skipped acceptor site.
[c] Difference in information content between used acceptor site and skipped acceptor site (see exon-specific values in Table 2). Positive values indicate that used site has higher information content whereas negative values indicated that skipped acceptor site had higher information content.
[d] Calculated fold difference in binding between used acceptor site and skipped acceptor site (see "Materials and Methods"). Values greater than the null value of 1 indicate fold improvement in predicted binding of used site over skipped site, whereas values <1 indicate fold decrease in predicted binding of used *versus* skipped site.

phenotype (31) that might provide a selective advantage in tumorigenesis. However, we found no evidence that *POLβ* is a frequent target for mutation in bladder tumors. In the course of screening these tumors we did detect a previously reported (27) polymorphism in codon 242 and a novel polymorphism of the exon 14 splice acceptor site that would be predicted to improve the acceptor site, but the functional consequences of both polymorphisms remain unknown.

With analysis of cDNA samples, an array of putative deletions, point mutations, and polymorphisms of *POLβ* have been reported in the literature (13–15, 28, 32). Some have questioned whether the frequent "deletions" reported might be splice variants although this had not been tested with direct comparison of cDNA and genomic DNA samples (33). There have been a number of reports suggesting these "deletions" as tumor-specific in breast, lung, and renal carcinomas (28, 34, 35). In addition, some reports of base sequence changes, described either as "mutations" or "polymorphisms," appear to have arisen because of differences in *POLβ* sequences deposited in GenBank (36) and may reflect mutations present in the cDNA reference sequence.

Whereas small deletions within *POLβ* are not found when using genomic DNA, we find that such deletions are common in cDNA from bladder tumor tissue, normal bladder tissue, and bladder cancer cell lines. These deletions all start and/or stop at exon-exon junctions, and given that these samples had normal full-length genomic DNA, establish the deletions as splice variants. Interestingly, splice variants outnumbered full-length cDNA in our sample of sequenced clones, and different splice variants were present in all of the samples examined, suggesting that multiple alternative splicing of *POLβ* is frequent in both tumor and normal tissue.

The basis for increased alternative splicing is not known. Alternative splicing of *POLβ* appears somewhat more frequently in tumor tissue and cell lines but is still a common event in normal tissue, suggesting that the high frequency of alternative splicing may be intrinsic in the sequence of this gene. Both splice site strength and accessory regulatory sequences dictate splice site use (37, 38). This is well demonstrated by our findings that in the majority of alternative splices the skipped upstream acceptor site had lower information content (R_i values) than that of ultimate downstream acceptor site. However, R_i values for alternatively spliced *POLβ* donor and acceptor sites are not outside of the normal range observed for other genes, and so the strengths of the alternatively spliced acceptor sites cannot alone explain the high frequency of alternative splicing for this gene relative to other genes.

Illegitimate alternative splicing has been suggested to result from abnormal stoichiometry of splicesome components (39), from decreased specificity of binding of splicing regulatory proteins because of the induction of alternative incomplete isoforms (40), and to ectopic induction (41) or increased expression (42) of splicing regulators. Experimental studies indicate that weak alternatively spliced acceptors may be regulated by genetic elements embedded in nearby coding and noncoding sequences (29). Purine-rich splicing enhancers in adjacent exons assist in recognition of weak acceptors by binding to serine and arginine-rich proteins, which stimulate the assembly of the presplicesomal factor U2AF to the polypyrimidine tract. By contrast, Sx1 and PTB/hnRNPI protein binding prevent use of the upstream site by competing with U2AF for occupancy of the polypyrimidine tract (43).

It is important to note that the genomic mutational analysis of *POLβ* included the donor and acceptor sites of each exon of *POLβ* and, with the exception of a polymorphism in the acceptor site of exon 14 (Fig. 1), we found no alterations. Thus, our data can provide no support for the hypothesis suggested previously that alterations in splicing consensus sequences are causing the alternative splicing of this gene (28).

Although dominant negative effects have been described for *POLβ* with the deletion of exon 11 (44), we found it only once and in a cell line. The absence of exon 11 has been reported in colorectal tumor (15, 33), testis tumor (33), lung tumor (28), normal blood samples of Werner patients (32), and normal colorectal tissue (15, 33), along with several cell lines (16). The functional activities of this and other *POLβ* splice variants remain largely unknown.

The majority of the splice variants described here are similar in that they involve the loss of exon 2 and the retention of exon 3. Interestingly, this pattern of exon 2 loss with exon 3 retention persists even in splice variants with multiple exon losses. These would be predicted to produce a 26 amino acid product that would include the first 20 amino acids of the single-stranded DNA binding dRPase domain of *POLβ* (45). If alternative splicing of *POLβ* is under cell control, it raises the intriguing and as yet untested hypothesis that the truncated protein might function as a dominant negative regulator of *POLβ* activity in normal cellular homeostasis.

ACKNOWLEDGMENTS

We thank Drs. Samuel Wilson for his kind contribution of the plasmid containing exon α and Robert Sobol for reading of an earlier version of the manuscript. We also thank Greg Solomon, Christine Johnson, and Mark Randolph for their help with sequencing.

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