Supplementary Figure, Tables and Materials & Methods

Two-Polymerase Mechanisms Dictate Error-Free and Error-Prone Translesion DNA Synthesis in Mammals

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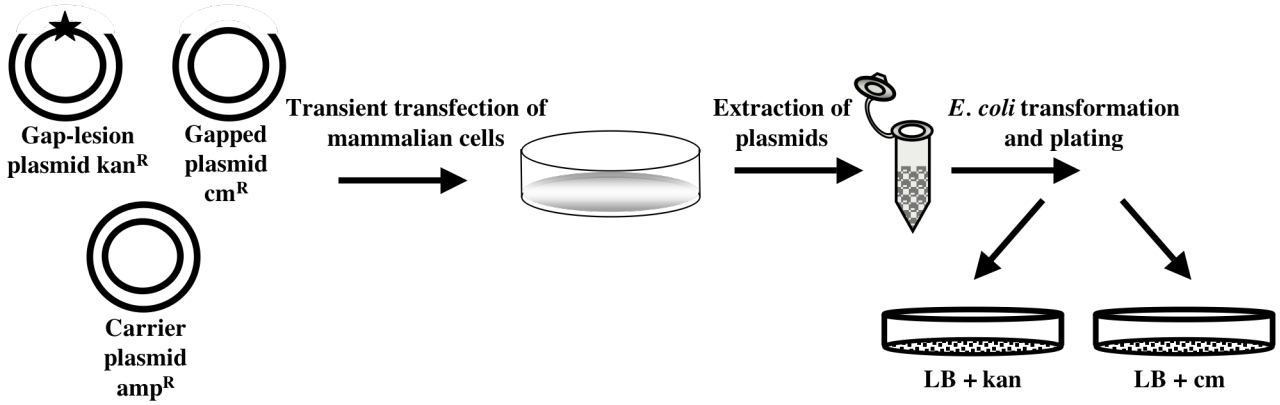

Figure 1s. Outline of the quantitative assay for TLS in cultured mammalian cells. Mammalian cells are transfected with a gap-lesion plasmid (kan^R) containing a site-specific lesion (indicated as a

black star), along with a gapped plasmid (cm^R) without a lesion, and a carrier plasmid (amp^R; pUC18). Following an incubation period the plasmids are extracted, and used to transform *E. coli* cells, which are then plated in parallel on kan-LB and cm-LB plates. The ratio of kan^R/cm^R transformants represents the extent of gap repair, which is mostly via TLS. Individual colonies are picked, and their plasmid contents analyzed for mutations in the DNA region corresponding to the original site of the gap. To obtain values of TLS from values of gap repair, the latter were multiplied by the percentage of TLS events out of the total events, as determined by the DNA sequence analysis.

Table 1s. TLS across TT CPD in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
0.5	42	290	16±1%	16±1%
1	181	510	38±1%	37±1%
1.5	237	465	52±5%	50±4%
2	91	133	57±2%	53±2%
4	101	127	72±6%	67±5%
6	77	93	80±15%	74±14%
8	319	296	93±4%	86±4%
10	237	242	93±8%	86±7%
24	476	432	93±2%	86±2%

The plasmid mixtures containing GP-TT-CPD (kan^R) along with the control GP20 (cm^R) and the carrier plasmids were introduced into U2OS cells by electroporation. Following incubation of 0-24h to allow TLS, the DNA was extracted and used to transform an *E. coli* indicator strain. Plasmid survival levels were calculated by the ratio of kan^R/cm^R colonies. TLS levels were calculated by reducing the relative ratio of non-TLS events (large insertions and deletions) from the corresponding plasmid survival values. Each point represents the average TLS level of four experiments.

Table 2s. TLS across BP-G in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
2	85	860	9±2%	9±2%
4	231	536	43±6%	42±6%
6	476	955	49±7%	48±6%
8	459	805	55±4%	55±4%
10	507	773	61±6%	60±6%
24	555	768	63±7%	63±7%

The experiment was performed as described in the legend to Table 1s, except that the gap-lesion plasmid was GP-BP-G (kan^R).

Table 3s. TLS across CisPt-GG in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
1	8	160	4±0.5%	4±0.5%
2	46	281	12±1%	12±1%
4	176	337	42±4%	41±4%
6	188	306	48±5%	47±5%
8	121	183	51±4%	50±4%
10	250	329	57±3%	56±3%
24	293	330	68±4%	67±4%

The experiment was performed as described in the legend to Table 1s, except that the gap-lesion plasmid was GP-CisPt-GG (kan^R).

Table 4s. TLS across TT 6-4 PP in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
1	11	227	1±1%	1±1%
2	44	503	6±4%	6±4%
4	144	845	13±2%	11±2%
6	231	1025	18±3%	16±3%
8	112	470	19±2%	18±1%
10	160	641	19±2%	17±2%
24	151	453	29±6%	27±5%

The experiment was performed as described in the legend to Table 1s, except that the gap-lesion plasmid was GP- 6-4 PP (kan^R).

Table 5s. TLS across an abasic (AP) site in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
2	67	462	8±3%	7±2%
4	63	341	14±2%	11±1%
6	103	449	18±3%	14±3%
8	145	572	20±1%	16±1%
10	137	450	23±4%	19±3%
24	141	432	30±2%	24±1%

The experiment was performed as described in the legend to Table 1s, except that the gap-lesion plasmid contained an AP site (kan^R).

Table 6s. TLS across 4-OHEN-C in human U2OS cells

TLS period (h)	Transformants		Plasmid repair	TLS
	Kan^R	Cm^R		
2	11	123	5±2%	3±1%
4	31	147	15±0.5%	9±0.5%
24	50	157	24±5%	14±3%

The experiment was performed as described in the legend to Table 1s, except that the gap-lesion plasmid was GP-4-OHEN-C (kan^R).

Table 7s. Analysis of mutations formed during TLS across a TT CPD in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
C-A-A-C	39 (84.8%)
G-A-A-C	1 (2.2%)
_-A-A-C	1 (2.2%)
C-**T**-A-C	2(4.3%)
Deletion/insertion	3 (6.5%)
Total clones analyzed	46 (100%)
Mutagenic TLS, %	9.3%

The experiment was performed as described in the legend to Table 1s using a plasmid mixture containing GP-TT CPD (kan^R). Plasmids were extracted from kan^R colonies containing GP-TT CPD descendants and subjected to DNA sequence analysis. The two bases opposite the dimer are underlined. Mutagenic TLS was calculated as the percentage of non-AA sequences inserted opposite the TT CPD out of all TLS events (which do not include large insertions or deletions).

Table 8s. Analysis of mutations formed during TLS across a BP-G in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
G-<u>C</u>-A	91 (86.7%)
G-<u>**A**</u>-A	6 (5.7%)
G-<u>**G**</u>-A	3 (2.9%)
G-<u>**T**</u>-A	4 (3.8%)
Deletion/insertion	1 (1%)
Total clones analyzed	105 (100%)
Mutagenic TLS, %	12.5%

The experiment was performed as described in the legend to Table 1s using a plasmid mixture containing GP-BP-G (kan^R). Plasmids were extracted from kan^R colonies containing GP-BP-G descendants and subjected to DNA sequence analysis. The base opposite the lesion is underlined. Mutagenic TLS was calculated as the percentage of non-C sequences inserted opposite the BP-G out of all TLS events (which do not include large insertions or deletions).

Table 9s. Analysis of mutations formed during TLS across a cisPt-GG in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
G-C-C-T	77 (86.5%)
G-**A**-C-T	7 (7.9%)
G-**T**-C-T	2 (2.2%)
G-C-**A**-T	1 (1.1%)
Deletion/insertion	2 (2.2%)
Total clones analyzed	89 (100%)
Mutagenic TLS, %	11.5%

The experiment was performed as described in the legend to Table 1s using a plasmid mixture containing GP-cisPt-GG (kan^R). Plasmids were extracted from kan^R colonies containing GP-cisPt-GG descendants and subjected to DNA sequence analysis. The two bases opposite the lesion are underlined. Mutagenic TLS was calculated as the percentage of non-CC sequences inserted opposite the cisPt-GG out of all TLS events (which do not include large insertions or deletions).

Table 10s. Analysis of mutations formed during TLS across a TT 6-4 PP in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
C-A-A-C	28 (23.3%)
A-A-A-C	1 (0.8%)
G-A-A-C	1 (0.8%)
T-**C**-A-C	1 (0.8%)
C-**G**-A-C	6 (5%)
C-**G**-**G**-C	1 (0.8%)
C-**G**-A-**A**	5 (4.2%)
C-**T**-A-C	3 (2.5%)
C-**T**-A-**A**	6 (5%)
C-_-A-C	1 (0.8%)
C-A-**C**-C	2 (1.7%)
C-A-**G**-C	2 (1.7%)
C-A-**T**-C	3 (2.5%)
C-A-**T**-**G**	1 (0.8%)
C-A-**T**-**T**	1 (0.8%)
C-A-_-C	3 (2.5%)
C-A-A-**A**	44 (36.7%)
Deletion/insertion	11 (9.2%)
Total clones analyzed	120 (100%)
Mutagenic TLS, %	74.3%

The experiment was performed as described in the legend to Table 1s using a plasmid mixture containing GP-6-4 PP (kan^R). Plasmids were extracted from kan^R colonies containing GP-6-4 PP descendants and subjected to DNA sequence analysis. The two bases opposite the dimer are underlined. Mutagenic TLS was calculated as the percentage of non-AA sequences inserted opposite the TT 6-4 PP out of all TLS events (which do not include large insertions or deletions).

Table 11s. Analysis of mutations formed during TLS across a 4-OHEN-C in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
C-<u>G</u>-C	16 (14.7%)
C-<u>**A**</u>-C	19 (17.4%)
C-<u>**C**</u>-C	12 (11%)
C-<u>**T**</u>-C	2 (1.8%)
C-<u>_</u>-C	10 (9.2%)
C-<u>_</u>-_	6 (5.5%)
Deletion/insertion	44 (40.4%)
Total clones analyzed	109 (100%)
Mutagenic TLS, %	75.4%

The experiment was performed as described in the legend to table 1s using a plasmid mixture containing GP-4-OHEN-C (kan^R). Plasmids were extracted from kan^R colonies containing GP-4-OHEN-C descendants and subjected to DNA sequence analysis. The base opposite the lesion is underlined. Mutagenic TLS was calculated as the percentage of non-G sequences inserted opposite the 4-OHEN-C out of all TLS events (which do not include large insertions or deletions).

Table 12s. Analysis of mutations formed during TLS across an AP site in U2OS cells

Nucleotide inserted opposite lesion	Number of isolates
C-<u>A</u>-C	73 (50.7%)
C-<u>C</u>-C	20 (13.9%)
C-<u>G</u>-C	3 (2.1%)
C-<u>T</u>-C	14 (9.7%)
C-<u>_</u>-C	6 (4.2%)
Deletion/insertion	28 (19.4%)
Total clones analyzed	144 (100%)

The experiment was performed as described in the legend to Table 1s using a plasmid mixture containing a gap-pleasmid with an AP site (kan^R). Plasmids were extracted from kan^R colonies containing and subjected to DNA sequence analysis. The base opposite the lesion is underlined.

Table 13s. Extent of TLS across various lesions in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

Cell line	Gap-lesion plasmid	Transformants		TLS, %
		Kan^R	Cm^R	
Rev3L $^{+/+}$	AP site	205	460	46.1±3.5%
Rev3L $^{-/-}$	AP site	98	506	22.6±2.8%
Rev3L $^{+/+}$	BP-G	59	194	27.6±6.8%
Rev3L $^{-/-}$	BP-G	59	1260	3.8±0.7%
Rev3L $^{+/+}$	cisPt-GG	41	193	21.3±5.8%
Rev3L $^{-/-}$	cisPt-GG	83	1228	6.1±2.5%
Rev3L $^{+/+}$	M12	23	270	3.9±1.2%
Rev3L $^{-/-}$	M12	28	393	0.5±0.1%
Rev3L $^{+/+}$	4-OHEN-C	99	314	33.1±7.1%
Rev3L $^{-/-}$	4-OHEN-C	103	832	11.9±2.9%
Rev3L $^{+/+}$	TT CPD	285	456	74.7±15.5%
Rev3L $^{-/-}$	TT CPD	521	670	75.5±13.2%
Rev3L$^{+/+}$	TT 6-4 PP	72	108	58.2±4.1%
Rev3L $^{-/-}$	TT 6-4 PP	53	265	10.9±3.6%

The cells were transfected with plasmid mixtures containing the indicated gap-lesion plasmid (kan^R) along with the control GP20 (cm^R). Following incubation of 24h to allow TLS, the DNA was extracted and used to transform an *E. coli* indicator strain. TLS extents were determined as described in the legend to Table 1s.

Table 14s. Analysis of mutations formed during TLS through a BP-G adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotide inserted opposite lesion	Number of isolates	
C	33 (62.3%)	34 (94.4%)
A	11 (20.7%)	2 (5.6%)
G	2 (3.8%)	-
T	7 (13.2%)	-
Deletion/insertion	1 (1.8%)	13 (26.5%)
Total clones analyzed	54 (100%)	49 (100%)
Mutagenic TLS, %	37.7%	5.6%
		P=0.0006 [1]

The experiment was performed as described in the legend to Table 13s using a plasmid mixture containing GP-BP-G (kan^R). Plasmids were extracted from kan^R colonies containing GP-BP-G descendants and subjected to DNA sequence analysis. Mutagenic TLS was calculated as the percentage of non-C sequences inserted opposite the BP-G out of all TLS events (which do not include insertions or deletions).

[1] *P* value is given for the difference in mutagenic TLS between *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs, and was calculated using the chi-square test.

Table 15s. Analysis of mutations formed during TLS across a cisPt-GG adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotides inserted opposite lesion	Number of isolates	
CC	47 (87%)	54 (100%)
CT	-	-
AA	2 (3.7%)	-
AC	-	-
CA	3 (5.5%)	-
AG	2 (3.7%)	-
Deletion/insertion	6 (10%)	4 (7%)
Total clones analyzed	60 (100%)	58 (100%)
Mutagenic TLS, %	13%	≤ 1.8%
		P =0.0062 [1]

The experiment was performed as described in the legend to table 13s using a plasmid mixture containing GP-cisPt-GG (kan^R). Plasmids were extracted from kan^R colonies containing GP-cisPt-GG descendants and subjected to DNA sequence analysis. Mutagenic TLS was calculated as the percentage of non-CC sequences inserted opposite the cisPt-GG out of all TLS events (which do not include insertions or deletions).

[1] P value is given for the difference in mutagenic TLS between *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs, and was calculated using the chi-square test.

Table 16s. Analysis of mutations formed during TLS across a 4-OHEN-C adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotide inserted opposite lesion	Number of isolates (%)	
G	6 (24%)	18 (78.3%)
A	17 (68%)	3 (13%)
T	2 (8%)	2 (8.7%)
Total mutants analyzed:	25 (100%	23 (100%)
Mutants, %	76%	21.7%
		P=0.0002

The experiment was performed as described in the legend to table 13s using a plasmid mixture containing GP-4-OHEN-C (kan^{R}). Plasmids were extracted from kan^{R} colonies containing GP-4-OHEN-C descendants and subjected to DNA sequence analysis. Mutagenic TLS was calculated as the percentage of non-G sequences inserted opposite the 4-OHEN-C out of all TLS events (which do not include insertions or deletions).

[1] *P* value is given for the difference in mutagenic TLS between *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs, and was calculated using the chi-square test.

Table 17s. Analysis of mutations formed during TLS across a TT 6-4 PP adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotide inserted opposite lesion	Number of isolates (%)	
AA	16 (40%)	32 (84%)
AAA	11 (27.5%)	-
GAA	2 (5%)	-
TAA	2 (5%)	-
AG	1 (2.5%)	-
AT	4 (10%)	-
AC	1 (2.5%)	1 (2.6%)
GA	1 (2.5%)	-
TT	1 (2.5%)	-
Deletion/insertion	1 (2.5%)	5 (13.4%)
Total mutants analyzed:	40 (100%)	38 (100%)
Mutagenic TLS, %	59%	3%
		P<0.0001

The experiment was performed as described in the legend to Table 13s using a plasmid mixture containing GP-6-4 PP (kan^R). Plasmids were extracted from kan^R colonies containing GP-6-4 PP descendants and subjected to DNA sequence analysis. Mutagenic TLS was calculated as the percentage of non-AA sequences inserted opposite the TT 6-4 PP out of all TLS events (which do not include insertions or deletions).

[1] P value is given for the difference in mutagenic TLS between *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs, and was calculated using the chi-square test.

Table 18s. Analysis of mutations formed during TLS across a TT CPD adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotide inserted opposite lesion	Number of isolates (%)	
AA	27 (100%)	27 (100%)
Total mutants analyzed:	27 (100%)	27 (100%)
Mutagenic TLS, %	<3.7%	<3.7%

The experiment was performed as described in the legend to Table 13s using a plasmid mixture containing GP-TT-CPD (kan^{R}). Plasmids were extracted from kan^{R} colonies containing GP-TT CPD descendants and subjected to DNA sequence analysis. Mutagenic TLS was calculated as the percentage of non-AA sequences inserted opposite the TT CPD (none in this case) out of all TLS events.

Table 19s. Analysis of mutations formed during TLS across an AP site adduct in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype:	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Nucleotide inserted opposite lesion	Number of isolates	
A	22 (67%)	22 (73%)
G	-	2 (7%)
T	4 (12%)	-
C	2 (6%)	-
-1 deletion	5 (15%)	6 (20%)
Total clones analyzed	33 (100%)	30 (100%)

The experiment was performed as described in the legend to Table 13s using a plasmid mixture containing a gapped plasmid with an AP site (kan^R). Plasmids were extracted from kan^R colonies and subjected to DNA sequence analysis.

Table 20s. Analysis of mutations formed during TLS across a 12 hydrocarbon chain (M12) in *Rev3L*$^{+/+}$ and *Rev3L*$^{-/-}$ MEFs

MEF genotype	*Rev3L*$^{+/+}$	*Rev3L*$^{-/-}$
Mutation type	Number of isolates (%)	
A	1 (3%)	-
C	1 (3%)	-
T	1 (3%)	-
AT	3 (9.5%)	1 (4%)
AAT	1 (3%)	-
TTT	1 (3%)	-
ATT	4 (12.5%)	-
GA	1 (3%)	-
Complex events	7 (22%)	-
-1 deletion	-	2 (7%)
Deletion/insertion	12 (38%)	25 (89%)
Total mutants analyzed:	32 (100%)	28 (100%)
		P<0.0001

The experiment was performed as described in the legend to Table 13s using a plasmid mixture containing GP-M12 (kan^R). Plasmids were extracted from kan^R colonies containing GP-M12 descendants and subjected to DNA sequence analysis. Complex events include multiple point mutations and small deletions.

[1] P value is given for the difference in TLS events (misinsertion or -1 deletion) between *Rev3L*$^{+/+}$ (40%) and *Rev3L*$^{-/-}$ (11%) MEFs, and was calculated using the chi-square test.

Table 21s. Extent of TLS in U2OS cells in which REV3L expression was knocked-down with siRNA

siRNA	Gap-lesion plasmid	Transformants		Plasmid repair, %	TLS, %	Relative TLS, %
		Kan^R	Cm^R			
Control	BP-G	169	1516	12.6±1%	10.9±1%	100
REV3L	BP-G	113	2764	4.2±1%	2.0±0.5%	18.3
Control	cisPt GG	57	305	21.2±3.3%	20.4±2.7%	100
REV3L	cisPt GG	24	471	6.1±2%	3.7±0.8%	18.1
Control	TT CPD	142	190	73.8±7%	73.8±7%	100
REV3L	TT CPD	201	369	55.6±6%	55.6±6%	75.3
Control	TT 6-4 PP	29	195	12.5±3%	7.2±2.9%	100
REV3L	TT 6-4 PP	16	224	5.9±1%	0.7±0.9%	9.7

U2OS cells were transiently transfected with *REV3L* siRNA. After incubation of 72h, the plasmid mixtures containing a gap-lesion plasmid (kan^R) along with the control GP20 (cm^R) and the carrier plasmids were introduced into the cells. Following incubation of 8h to allow TLS, the DNA was extracted and used to transform an *E. coli* indicator strain. Plasmid survival levels were calculated by the ratio of kan^R/cm^R colonies. TLS levels were calculated by reducing the relative ratio of non-TLS events (large insertions / deletions) from the corresponding plasmid survival values. Each point represents the average TLS level of at least four experiments.

Table 22s. Extent of TLS across BP-G in human U2OS cells in which the expression of specific TLS DNA polymerases was knocked-down using siRNA.

siRNA	Gap-lesion plasmid	Transformants		Plasmid repair, %	**TLS, %**	**Relative TLS, %**
		Kan^R	Cm^R			
Control	GP-BPG1	169	1516	12.6±1	**10.9±1.1**	**100**
REV3L	GP-BPG1	113	2764	4.2±1	**2.0±0.5**	**18.3**
POLK	GP-BPG1	87	1062	6.8±1	**4.7±0.7**	**43.1**
POLH	GP-BPG1	98	1040	10.6±2	**8.0±1.2**	**73.4**
POLK + *REV3L*	GP-BPG1	117	2932	3.7±1	**1.8±0.5**	**16.5**
POLK + *POLH*	GP-BPG1	97	1566	7.1±0.3	**4.5±0.2**	**41.3**

U2OS cells were transiently transfected with the indicated polymerase-specific siRNAs. After incubation of 72h, the plasmid mixtures containing the gap-lesion plasmid GP-BP-G1 (kan^R) along with the control gapped plasmid GP20 (cm^R) and the carrier plasmid were introduced into the cells. Following incubation of 8h to allow TLS, the DNA was extracted and used to transform a *recA E. coli* indicator strain. Plasmid survival levels were calculated by the ratio of kan^R/cm^R colonies. TLS levels were calculated by multiplying the plasmid repair extent by the fraction of TLS events (i.e., excluding large deletions and insertions) as determined by DNA sequence analysis of individual clones (shown in Table 1). Each point represents the average TLS level of 4-7 experiments.

Table 23s. Extent of TLS across cisPt-GG in human U2OS cells in which the expression of specific TLS DNA polymerases was knocked-down using siRNA.

siRNA	Gap-lesion plasmid	Transformants		Plasmid repair, %	**TLS, %**	**Relative TLS, %**
		Kan^R	Cm^R			
Control	GP-cisPt-GG	57	305	21.2±3.3	**20.4±2.7**	**100**
REV3L	GP-cisPt-GG	24	471	6.1±2	**3.7±0.8**	**18.1**
POLK	GP-cisPt-GG	96	640	15±3.7	**13.4±3**	**65.7**
POLH	GP-cisPt-GG	84	1222	10.9±1.1	**9.5±0.6**	**46.6**
POLH + *REV3L*	GP-cisPt-GG	76	1821	6.2±2	**4±0.6**	**19.6**
POLK + *POLH*	GP-cisPt-GG	82	1840	5.8±0.3	**4.6±0.2**	**22.5**

U2OS cells were transiently transfected with the indicated polymerase-specific siRNAs. After incubation of 72h, the plasmid mixtures containing the gap-lesion plasmid GP-cisPt-GG (kan^R) along with the control gapped plasmid GP20 (cm^R) and the carrier plasmid were introduced into the cells. Following incubation of 8h to allow TLS, the DNA was extracted and used to transform a *recA E. coli* indicator strain. Plasmid survival levels were calculated by the ratio of kan^R/cm^R colonies. TLS levels were calculated by multiplying the plasmid repair extent by the fraction of TLS events (i.e., excluding large deletions and insertions) as determined by DNA sequence analysis of individual clones (shown in Table 1). Each point represents the average TLS of 4-7 experiments.

Supplementary Materials and Methods

Construction of DNA substrates and plasmids

The DNA sequences in the vicinity of the gap-lesion structure of the gap-lesion plasmids used in this study were as follows (the lesions are in bold face type and underlined):

AP site
5'-CAACGAAGTGATTCCCGTCGTGACTG**X**GAAAACCCTGGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-BP-G1
5'-CAACGAAGTGATTCCGGCAT**G**CGTCCTACCTGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-cisPt-GG
5'-CAACGAAGTGATTCCTCTCTA**GG**CCTTCTGAGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-M12
5′-CAACGAAGTGATTCCCGACGCGAGAA**X**GTCAACCCTGGGCTACTTGAACCAG-3′
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-4-OHEN-C
5'-CAACGAAGTGATTCCTCGGTAG**C**GATGGTCGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-TT-CPD
5'-CAACGAAGTGATTCCTCGCAAG**TT**GGAGCTGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

GP-TT-6-4PP
5'-CAACGAAGTGATTCCTCGCAAG**TT**GGAGGAGGCTACTTGAACCAG-3'
3'-GTTGCTTCACTAAGG-5' 3'-CCGATGAACTTGGTC-5'

We have previously described the construction of gapped plasmids containing a site-specific synthetic AP site (Reuven et al., 1999), (+)–*trans*-BPDE-N^2-dG adduct (Avkin et al., 2004), a dodecamethylene chain, M12 (Maor-Shoshani et al., 2003), cisplatin-GG (Avkin et al., 2006)), and TT CPD or TT 6-4 PP (Hendel et al., 2008). A gap-lesion plasmid with a site-specific 4-OHEN-C adduct, derived from the

reaction of 4-hydroxyequilenin (4-OHEN) with the single C residue in the 11-mer oligonucleotide 5'-GGTAGCGATGG-3' was generated as previously described (Kolbanovskiy et al., 2005). The structure of the 4-OHEN-C adduct was elucidated by Shen et al. (Shen et al., 1997). It was extended to a 53-mer oligonucleotides by ligating to its 5' end the 21-mer 5'-ACCGCAACGAAGTGATTCCTC-3', and to its 3' the 21-mer 5'- CTGGCTACTTGAACCAGACCG-3', using as a scaffold the 34-mer 5'- AAGTAGCCAGCCATCGCTACCGAGGAATCAC-3'. The resulting 53-mer was separated from the scaffold and excess 21-mers on a 12% denaturing polyacrylamide gel containing 8 M urea, and used to construct the gap-lesion plasmid GP-4-OHEN-C. We have also prepared gap-lesion plasmids with gaps starting 11 nucleotides upstream the lesion (an abasic site or BP-G), and extending 200-350 nucleotides past the lesion, as previously described (Tomer et al., 1998). TLS with these substrates was the same as with the corresponding substrates with the small gaps (data not shown).

RT-PCR and immunoblot analysis

Total RNA was extracted from cells using the RNeasy mini kit from Qiagen. Total RNA (1μg) was used for cDNA Synthesis and RT-PCR (Access RT-PCR system, Promega) using the primers 5'-ACCACAGTCCATGCCATCAC-3' and 5'-TCCACCACCCTGTTGCTGTA-3' for the *GAPDH* gene; 5'-GCTAAGAGGCTGTGCCCACAA-3' and 5'-TCACTGAATGTCCTCTCAACG-3' for the *hPOLK* gene; 5'-GCGGTGACAGCCACTAAGAA-3' AND 5'-GCGTTTATTAGTGCAGGCCAA-3' for the *hPOLH* gene, and the primers 5'-AAGGAGCCACTAAGGAGCAG-3' and 5'-GATGACGTATGGCACTCG-3' common to the human *REV3L* and mouse *Rev3L* genes. RT-PCR was performed by 24 cycles of 1 min at 94°C, 45 sec at 60°C, and 45 sec at 68°C. Immunoblot analysis of polη was performed with a total of 40 μg whole cell lysate, using a mouse monoclonal anti-polη antibody (Santa Cruz-17770).

Cell cultures.

Rev3L$^{-/-}$ and *Rev3L*$^{+/+}$ mouse embryonic fibroblasts were generated as previously described (Wittschieben et al., 2006). They were both pol iota-deficient, with the 129 strain mutation, as verified by PCR analysis. *Rev3L*$^{-/-}$ MEFs and U2OS and XP12RO (an SV40-transformed cel line from an XPA patient) cells were maintained in Dulbeco's Modified Engle's Medium (DMEM) supplemented with 2 mM glutamine (GIBCO/BRL). The medium was supplemented with 10% fetal bovine serum (Hyclone), 100 units/ml of penicillin and 100 μg/ml of streptomycin (Biological Industries). The cells were incubated at 37^{o}C in a 5% CO_2 atmosphere. The doubling time of the U2OS cells was 24 h.

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