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Short communication

# DNA sequence context greatly affects the accuracy of bypass across an ultraviolet light 6-4 photoproduct in mammalian cells

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ABSTRACT

Translesion DNA synthesis (TLS) is a DNA damage tolerance mechanism carried out by low-fidelity DNA polymerases that bypass DNA lesions, which overcomes replication stalling. Despite the miscoding nature of most common DNA lesions, several of them are bypassed in mammalian cells in a relatively accurate manner, which plays a key role maintaining a low mutation load. Whereas it is generally agreed that TLS across the major UV and sunlight induced DNA lesion, the cyclobutane pyrimidine dimer (CPD), is accurate, there were conflicting reports on whether the same is true for the thymine–thymine pyrimidine–pyrimidone(6-4) ultraviolet light photoproduct (TT6-4PP), which represents the second most common class of UV lesions. Using a TLS assay system based on gapped plasmids carrying site-specific TT6-4PP lesions in defined sequence contexts we show that the DNA sequence context markedly affected both the extent and accuracy of TLS. The sequence exhibiting higher TLS exhibited also higher error-frequency, caused primarily by semi-targeted mutations, at the nearest nucleotides flanking the lesion. Our results resolve the discrepancy reported on TLS across TT6-4PP, and suggest that TLS is more accurate in human cells than in mouse cells.



## 1. Introduction

DNA repair mechanisms are conserved in essentially all living organisms, and function to preserve the accuracy of genetic information [1]. Yet the high abundance of DNA damage, caused by both intracellular (e.g., reactive oxygen species) and external (e.g., sunlight, tobacco smoke) agents, estimated at thousands per day, make the encounter of replication with unrepaired damages inevitable [2,3]. When this happens, cells utilize DNA damage tolerance mechanisms, which function to bypass the lesion without actually removing it from DNA. Two DNA damage tolerance mechanisms are known. Translesion DNA synthesis (TLS) utilizes specialized low-fidelity DNA polymerases to bypass the lesions, whereas homology-dependent repair (HDR, also termed postreplication repair, damage avoidance, or template switch) utilizes the homologous sister chromatid as a DNA sequence source for bypassing the lesion [2–4]. While TLS is inherently inaccurate due to the miscoding of most DNA lesions [5,6], HDR is fundamentally accurate [7–10]. Remarkably, despite the great heterogeneity of DNA lesions, TLS often incorporates the correct nucleotide(s) opposite it, leading to accurate TLS [5]. The classical example is the UV and sunlight-induced TT cyclobutane pyrimidine dimer (CPD), which is bypassed by DNA polymerase η (polη) with an accuracy of 99% [11,12]. Similarly, the bulky benzo[*a*]pyrene-guanine adduct is bypassed with accuracy of nearly 90% in human cells [13].

Short wavelength UV light produced in DNA two main types of lesions: CPD, mentioned above, which account for 66–75% of the photoproducts, and pyrimidine–pyrimidone (6-4) photoproducts (6-4PP), which account for 25–33% of the photoproducts [1]. The 6-4PP are efficiently repaired by nucleotide excision repair, because they cause a strong distortion in DNA [14], whereas the CPD, which cause a mild distortion in DNA, are poorly repaired, and are dealt with primarily during replication via TLS by polη [15,16]. Yet, replication may encounter also unrepaired 6-4PP, a situation in which TLS functions to enable completion of replication. The TT6-4PP lesion was initially reported to be highly mutagenic [17,18], later confirmed by studies from our group [13,19]. Yet, a publication from the Prakash group reported that this lesion is accurately bypassed in mammalian cells, and suggested that the difference stems from the fact that they assayed replicative TLS in a plasmid,

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whereas we used gap-filling TLS in a non-replicating plasmid [20]. An alternative explanation for the differences observed in the accuracy of TLS in these studies is the DNA sequence context of the lesion. To explore this possibility we examined TLS across a site-specific single TT6-4PP in the sequence context used in the Prakash study to the one used in our study. We found that the DNA sequence context had a major effect on the accuracy of TLS in mammalian cells, and that overall it was more accurate in human cells than in mouse cells.

## 2. Materials and methods

### 2.1. Construction of DNA substrates and plasmids

The construction of the control gapped plasmid GP20 was previously described [21–23]. A gap-lesion plasmid with a site-specific TT6-4PP flanked by sequences used previously in the gap-lesion plasmid system, was prepared using the site specifically modified 11-mer oligonucleotide 5′-GCAAGTTGGAG-3′ containing a single site-specific TT 6-4 PP (underlined) as previously described [19]. Briefly, the 11-mer was extended to a 53-mer oligonucleotide by ligating to its 5′ end the 21-mer 5′-ACCGCAACGAAGTGATTCCTC-3′, and to its 3′ the 21-mer 5′-GAGGCTACTTGAACCAGACCG-3′, using as a scaffold the 33-mer 5′- AAGTAGCCTCCTCCAACTTGCGAGGAATCAC-3′. The 53-mer was separated from the scaffold and excess 21-mers on a 12% denaturing polyacrylamide gel containing 8 M urea, and then annealed to the two complementary 15-mers 5′-GGAATCACTTCGTTG-3′ and 5′-CTGGTTCAAGTAGCC-3′. The resultant gapped duplex oligonucleotide, with the lesion opposite the gap, was used to construct GP-TT6-4PP-L-seq. A gap-lesion plasmid with a site-specific TT6-4PP flanked by sequences used previously in the gap free-lesion plasmid replicative system, was similarly prepared, except that the 11-mer oligonucleotide was 5′-AGCAATTGTAC-3′ containing a single site-specific TT6-4PP (underlined), and it was extended 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 33-mer 5′- AAGTAGCCAGGTACAATTGCTGAGGAATCAC-3′.

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 underlined):

GP-TT6-4PP-L-seq:

5′CAACGAAGTGATTCCTCGCAAGTTGGAGGAGGCTACTTGAACCAG3′
3′GTTGCTTCACTAAGG CCGATGAACTTGGTC5′

GP-TT6-4PP-P-seq:

5′CAACGAAGTGATTCCTCAGCAATTGTACCTGGCTACTTGAACCAG3′
3′GTTGCTTCACTAAGG CCGATGAACTTGGTC5′

### 2.2. TLS assay

To examine TLS across the TT6-4PP in different sequence contexts we used the gap-lesion plasmid assay, developed in our laboratory more than a decade ago [24], and extensively utilized since to study TLS [22,25–27]. The system is based on a plasmid carrying a site-specific lesion, engineered in a particular sequence context, and opposite a single-stranded gap. When such a plasmid is used to transfect mammalian cells (Fig. 1), the gap is filled in by the cellular TLS machinery, after which product covalently closed plasmids are extracted, and introduced for analysis in an indicator *Escherichia coli recA* strain, which is TLS-deficient. This enables measurement of the efficiency of TLS by counting the number of *E. coli* colonies, and its accuracy, by DNA sequence analysis of plasmids extracted from individual colonies (Fig. 1). The system assays TLS of gap filling in the absence of DNA replication, and was proven to depend on the DNA polymerase composition of the cells [19,28],

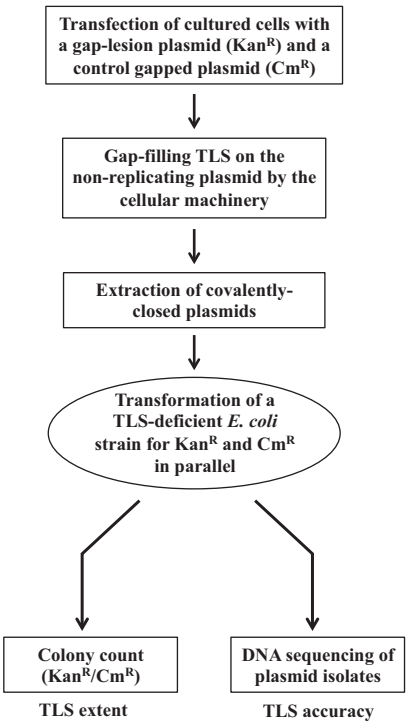


**Fig. 1.** Flow-chart outlining the gap-lesion plasmid-based TLS assay. Kan$^R$, kanamycin resistance; Cm$^R$, chloramphenicol resistance. See text for details.

and on regulators of TLS such as ubiquitination of PCNA [22] and on the cell cycle [26].

The TLS assay was executed as previously described [23,25], using SV40-transformed MRC5sv human fetal lung fibroblasts [27] or mouse embryo fibroblasts [26]. Briefly, transfection was done in 6-well plates (surface area 9.1 cm$^2$) at ~80% confluence using Jet-PEI transfection reagent. After allowing time for TLS (~20 hours), the cells were harvested and plasmids were extracted using a plasmid extraction kit HiYield$^{TM}$ Plasmid Mini Kit/96-well Plasmid Kit/Real Genomics$^{TM}$. Plasmid DNA extraction was done under alkaline lysis conditions followed by renaturation, which leaves only covalently closed plasmids (namely those which underwent complete gap-filling TLS) non denatured. The extracted plasmids were used for transfection of competent *E. coli* JM109 *recA* cells. The percentage of plasmid repair, of which most occurs by TLS across TT6-4PP was calculated by dividing the number of colonies obtained from the descendants of gap-lesion plasmids (Kan$^R$ colonies) by the number of colonies obtained from descendants of the control gapped plasmids (Cm$^R$ colonies). The DNA sequence opposite the lesion was determined using Sanger sequencing. A small fraction of gap-lesion plasmids was repaired by non-TLS events, which most likely involve formation of a double stranded break as an intermediate. These events were observed as large deletions or insertions in the plasmid isolates. To obtain precise TLS extents, the plasmid repair extents were multiplied by the fraction of TLS events out of all plasmid repair events, as obtained by DNA sequence analysis.

## 3. Results

### 3.1. The extent of TLS is sequence context dependent

Using the gapped plasmid assay we assayed TLS across TT6-4PP in the two DNA sequence contexts in human MRC5sv cells. As can be seen in Table 1, TLS across the TT6-4PP in sequence context L-seq amounted to 37%. In contrast, TLS across the same lesion in