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Multiple two-polymerase mechanisms in mammalian translesion DNA synthesis

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Key words: translesion synthesis, TLS, XPV, DNA repair, DNA polymerase, carcinogenesis, cancer

Abbreviations: BPDE, benzo[*a*]pyrene diol epoxide; BP-G, benzo[*a*]pyrene-guanine adduct; cisPt-GG, cisplatin-guanine-guanine adduct; CPD, cyclobutane pyrimidine dimer; HDR, homology-dependent repair; NER, nucleotide excision repair; pol, DNA polymerase; TLS, translesion DNA synthesis

The encounter of replication forks with DNA lesions may lead to fork arrest and/or the formation of single-stranded gaps. A major strategy to cope with these replication irregularities is translesion DNA synthesis (TLS), in which specialized error-prone DNA polymerases bypass the blocking lesions. Recent studies suggest that TLS across a particular DNA lesion may involve as many as four different TLS polymerases, acting in two-polymerase reactions in which insertion by a particular polymerase is followed by extension by another polymerase. Insertion determines the accuracy and mutagenic specificity of the TLS reaction, and is carried out by one of several polymerases such as polη, polκ or polι. In contrast, extension is carried out primarily by polζ. In cells from XPV patients, which are deficient in TLS across cyclobutane pyrimidine dimers (CPD) due to a deficiency in polη, TLS is carried out by at least two backup reactions each involving two polymerases: One reaction involves polκ and polζ, and the other polι and polζ. These mechanisms may also assist polη in normal cells under an excessive amount of UV lesions.

Introduction

DNA damage is abundant, and formed in human cells at an estimated rate of 20,000/genome/day. It is formed by endogenous processes, such as depurination and reaction with reactive oxygen species, and by external agents such as radiation and pollutants.[1] DNA damage interferes with DNA replication and transcription, and if not repaired may cause mutations and cell death, leading to a multitude of severe diseases. Indeed, most DNA damage is removed from DNA prior to replication by error-free DNA repair mechanisms.[1] However, the encounter of replication forks with unrepaired lesions seems inevitable, since DNA repair does not eliminate all DNA damage prior to replication on one hand, and DNA damage continues to form during the replication, on the other hand. The molecular events occurring when a replication fork encounters an unrepaired DNA lesion are poorly understood, however, they lead to the formation of single-stranded DNA gaps, most probably due to arrest of the replicative DNA polymerase at lesions, followed by downstream re-initiation. Alternatively, the fork may be arrested at the lesion.[2-6] In each of these cases the error-free excision repair mechanisms, which rely on the complementary strand, cannot act to remove the lesion, since it is located on single-stranded DNA. To resolve this situation, and enable completion of DNA replication, DNA damage tolerance mechanisms bypass the lesion, without removing it from DNA, leading to restoration of the double-stranded structure of DNA. There are two universal strategies to tolerate DNA lesions: Translesion DNA synthesis (TLS), and homology-dependent repair (HDR). In TLS specialized low-fidelity DNA polymerases perform DNA synthesis across the lesion, a process which is inherently mutagenic due to the miscoding nature of most DNA lesions. In HDR, the undamaged and fully replicated sister chromatid serves as a source for filling the gap across the blocking lesion. This can occur by physical transfer of the complementary strand, a process termed homologous recombination repair (HRR), or strand transfer HDR. Alternatively the homologous strand can be copied, a process termed template switch HDR (or copy choice).[1,7] Unlike TLS, both versions of HDR are inherently error-free. It is therefore perhaps no wonder that HDR appears to predominate over TLS in tolerating DNA damage in *E. coli*,[8,9] in the yeast *S. cerevisiae*,[10,11] and is prominent in chicken DT40 cells.[12] In mammals the labor division between TLS and HDR is unknown. In fact, while HDR operates during the repair of double-strand breaks (DSB) in mammalian cells[13,14] there is currently no definitive evidence for the activity of HDR in tolerating replication blocking DNA lesions. In a first step toward clarifying the situation we have recently shown using a plasmid model assay system that in mammalian cells gaps opposite an abasic site or a BP-G (benzo[*a*]pyrene-guanine) adduct are filled in via a mechanism involving strand transfer HDR (HRR).[15] Still, the extent to which this and other HDR mechanisms act in mammalian chromosomes is not known. In contrast, it is well established that TLS is important in mammals. This is indicated by at least three lines of evidence: (1) the lack of DNA polymerase η (polη), the main TLS polymerase that bypasses UV-induced cyclobutane pyrimidine dimers (TT CPD), causes the severe hereditary

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disease xeroderma pigmentosum variant (XPV), which is characterized by sunlight sensitivity, and extreme predisposition to skin cancer;[16,17] (2) mammals contain at least five DNA polymerases that function in TLS, and perhaps several more;[18] (3) the *Rev3L* gene, encoding the catalytic subunit of the TLS DNA polymerase polζ is essential for life in mice.[19-21]

How Can the Mutational Load of a Mutagenic Repair Process Be Mitigated?

Why would mammalian cells contain multiple mutagenic DNA polymerases, which at least in theory might increase the risk of a malignant transformation? Since the chemistry spectrum of DNA damages is very broad, multiple polymerases may have evolved to carry out better bypass of specific cognate lesions. "Better" bypass ideally means a high extent of bypass, with low error frequency. This task is essentially impossible for a single general TLS polymerase, given the wide diversity of DNA damage. Specific polymerases might have evolved only for certain lesions which have been evolutionary important, such as sunlight-induced CPD, and which are not effectively removed by error-free repair mechanisms. For such a system to be effective in lowering the mutation burden, proper regulation is required, to make sure that the right polymerase gets to its target lesion at the right time. In addition to these specialized TLS pathways a general TLS pathway is also required, to deal with those lesions for which specialized cognate polymerases do not exist, e.g., uncommon lesions, or lesion that have appeared late in evolution. In other words, our view is that the multiplicity of polymerases enables specialization for particular types of DNA damage, each bypassed more effectively and more accurately by a specific cognate polymerase, and under the appropriate regulation, this would lead to an overall lower mutational load.[22]

Numerous studies with purified polymerases have established that there is a certain degree of lesion specificity for each DNA polymerase.[23-26] The best example is the bypass of a TT CPD by polη, which is very efficient and relatively accurate.[16,27,28] However, due to the overlap in lesion specificity of purified TLS polymerases, and the potential effect of accessory proteins, such a question must be examined in live cells. There are at least three examples in which TLS across a certain lesion is carried out by a specific TLS DNA polymerase more effectively and more accurately than by any other polymerase in mammalian cells. These are the bypass by polη of the UV light induced TT CPD,[29,30] the bypass by polκ of the tobacco smoke- and combustion-induced DNA adduct BP-G,[31-33] and the bypass by polη of the intrastrand cisplatin-GG adduct (cisPt-GG),[33,34] generated by the chemotherapeutic drug cisplatin. This assignment is based on experiments using gapped plasmids carrying site-specific lesions, which directly and quantitatively assay TLS in human and mice cells,[30,32,33] and on the observations of increased sensitivity and decreased mutagenesis upon treatment with UV, BPDE (benzopyrene-7,8-diol-9,10-epoxide) or cisplatin of cells deficient in polη,[29] polκ[31] and polη,[34] respectively. CPD and BP-G are evolutionary important lesions, and this may explain the evolution of TLS polymerases that target them. In contrast, cisPt-GG is likely an "artificial" lesion, and likely a relatively new adduct, whose effective and accurate bypass by polη might be a coincidence caused by its slight similarity to a CPD.

DNA Polymerase ζ Is a Master TLS Polymerase, Which Is Involved in the Bypass of a Very Broad Spectrum of Lesions

"Winner" lesion-polymerase combinations cannot apply for all DNA lesions, simply because those by far outnumber DNA polymerases. Thus, in many situations a general TLS pathway is employed, which is common to many types of DNA lesions. Such a pathway is likely to be more mutagenic than the specialized pathways, which is the price for covering a wide range of DNA lesions. A systematic analysis of seven very different types of DNA damage revealed that polζ was involved in TLS across all but the TT CPD.[33] In fact, under certain conditions polζ was involved also in the bypass of TT CPD (see below). Thus, it appears that polζ is a general master TLS polymerase, which handles a very broad spectrum of lesions. This is highlighted by the bypass of an artificial lesion consisting of a dodecamethylene chain -$(CH_2)_{12}$-, inserted into the backbone of a ssDNA stretch in a gapped plasmid. We used this "lesion" as an extreme and potentially novel challenge to DNA, as it is composed of a non-DNA hydrocarbon chain.[35] Strikingly mammalian cells are capable of TLS across this non-DNA segment (although with low efficiency), with the "misinsertion" of 2–3 nucleotides (the length of the insert is equivalent to the length of about 2.5 nucleotides).[36] This TLS was totally dependent on polζ, consistent with its central role in a general TLS pathway.[33]

Does polζ function only in mutagenic TLS, in case of lesions for which no specialized polymerase exists? TLS across two of the lesions that involves polζ, the UV light-induced TT 6-4 photoproduct (TT 6-4 PP), and the 4-hydroxyequilenin-C (4-OHEN-C), an adduct formed in DNA by metabolites of equilin and equilenin, which are widely used in estrogen replacement therapy, is largely mutagenic, with error frequencies of 60% and 75%, respectively.[33] In addition, TLS across an abasic site was highly mutagenic, although in the absence of a template base it is impossible to define which insertion is accurate.[33] However, TLS across cisPt-GG and BP-G in human cells, both involving polζ, is largely error-free, with an error frequency of about 10–15%.[33] Thus, in mammals polζ is involved in both relatively accurate ("error-free") and mutagenic ("error-prone") TLS. Interestingly, for all lesions that were examined, the relatively accurate TLS occurred with faster kinetics than the highly mutagenic TLS.[33]

Three DNA Polymerases Are Involved in TLS Across cisPt-GG in Human Cells

The fact that polζ is involved in TLS across BP-G and cisPt-GG, where 'champion' cognate DNA polymerases, polκ and polη, respectively, are involved, is somewhat surprising, since in vitro these polymerases can carry out complete bypass on their own. Why would then in vivo TLS require also polζ? In the absence

of data on the purified mammalian polζ protein, the properties of its *S. cerevisiae* homolog provide a useful model, although it should be kept in mind that the mammalian polζ is twice the size of the yeast polζ.[37] A key property of the *S. cerevisiae* polζ is its ability to efficiently extend mismatched primers, or primers located opposite lesions.[38,39] This property was at the basis of the two-polymerase model for TLS, according to which TLS is carried out in two steps, each carried out by a different DNA polymerase: Insertion, in which a nucleotide is placed opposite the lesion, and extension, in which the primer is extended past the lesion.[39] Furthermore, since also purified human polκ exhibited extender properties,[40] it was suggested that both polκ and polζ act as the extenders. Is this the case also in living cells?

We addressed this question using an siRNA epistasis analysis, in which TLS across cisPt-GG was assayed in human cells in which the expression of one or more TLS polymerases was knocked down. Knocking down the expression of *REV3L*, encoding the catalytic subunit of polζ, caused a dramatic five-fold decrease in the extent of TLS across cisPt-GG. Knocking down the expression of *POLH* (encoding polη), or *POLK* (encoding polκ) had each a mild effect on TLS extent; However, when the expression of both *POLK* and *POLH* was simultaneously knocked-down, TLS extent decreased by 5-fold, similar to the effect of polζ.[33] This implies that polζ cooperates with either polη or polκ to carry out TLS, and that the three are responsible for most of TLS across cisPt-GG. It also argues that despite its in vitro extender properties, polκ cannot backup polζ under these conditions. The simplest model to explain these results is that cisPt-GG is bypassed in two-polymerase reactions, in which insertion is carried out by polη or polκ, and extension is carried out by polζ (**Fig. 1**).

How about accuracy? Generally cisPt-GG is bypassed relatively accurately, with the correct CC nucleotides inserted in about 85% of the events.[33] Interestingly, the accuracy of polη and polκ in the insertion step is different: Almost all TLS errors across cisPt-GG were caused by polκ, whereas polη performed almost only accurate bypass.[33] Thus, three polymerases are involved in TLS across cisPt-GG, and the identity of the polymerases in these two-polymerase reactions determines the mutagenic outcome of the TLS reaction. Are additional polymerases involved? Based on the TLS data, if there are additional polymerases, their contribution to TLS is small.

Four DNA Polymerases Are Involved in TLS Across BP-G in Human Cells

A similar epistasis analysis was performed with the lesion BP-G. Similar to the case with cisPt-GG, knocking down the expression of polζ caused a drastic more than five-fold decrease of TLS, whereas knocking down either polκ or polη caused only a moderate decrease in TLS, which was stronger for polκ.[33] However, unlike in the case of cisPt-GG, knocking down the expression of *POLH* and *POLK* combined had an effect similar to that of *POLK* alone.[33] This suggests that an additional polymerase is involved in TLS across BP-G, presumably in the insertion step, and all the three cooperate with polζ, which maintains its key role as the putative major extender for this lesion too. Thus, at least four polymerases are involved in TLS across BP-G: polκ, polη polζ and an additional unknown polymerase. Of the four, polη was much more mutagenic at BP-G than polκ: When the expression of *POLH* was knocked-down, TLS across BP-G decreased only marginally by 27%, but the fraction of mutagenic TLS decreased by 68%. Polζ was involved in at least 80% of all TLS events, being responsible for both accurate and mutagenic TLS[33] (**Fig. 1**).

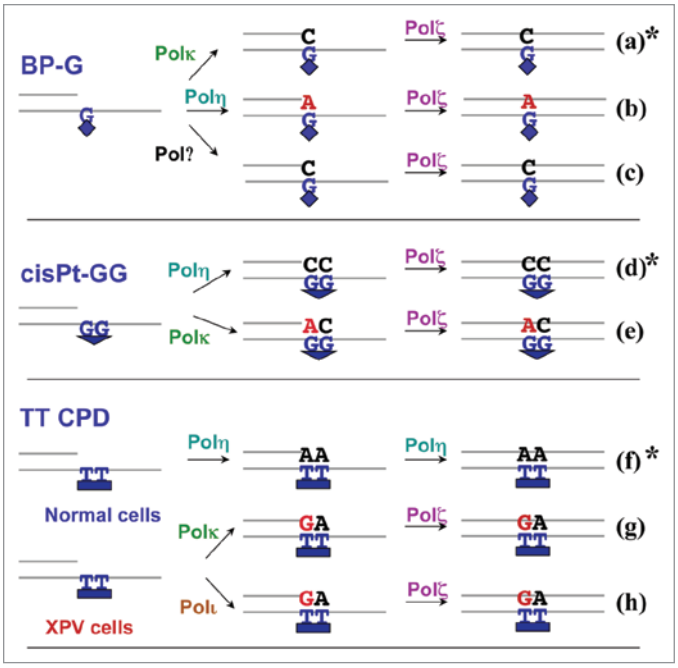

Figure 1. Model for TLS by two-polymerase reactions across several DNA lesions. The asterisks indicate the main reactions for each lesion. See text for details.

Is One DNA Polymerase Enough to Bypass a TT CPD?

Is the bypass of each lesion a multi-polymerase affair? Not necessarily. It is possible that some lesions are bypassed by a single polymerase, performing both insertion and extension. This may be the case of the TT CPD, which is bypassed effectively and relatively accurately by polη. In this case, the specialization of polη for bypassing a TT CPD is remarkable, since its catalytic efficiency in bypass of a TT CPD is in fact better than a non-modified TT sequence.[41] This TLS occurs in vitro with an error frequency of approximately 1%.[27,28,41] Does this occur also in vivo? Analysis of TLS across a site-specific TT CPD revealed 2 mutagenic events out of 66 (3%) in the Burkitt lymphoma cell line BL2, and 1 out of 85 events (1.2%), in SV40-transformed human fibroblasts (overall: 3/229 = 1.3%).[30] When primary normal fibroblasts were analyzed, no mutation was found out of 78 events (<1%).[30] Thus, the in vivo error frequency is similar to that observed in vitro, and in fact may be even better, since accuracy in normal primary cells might be better than in transformed or cancer cell lines. Does this mean that polη is the only

polymerase required for TLS across TT CPD? The answer to this question is currently unknown; however, knocking down polζ, which is involved in the bypass of a very large range of lesions had no or little effect on TLS across a TT CPD.[33] This combined with biochemical properties of polη suggests that at TLS across TT CPD polη alone is sufficient, although there might be instances when additional polymerases are involved (see below).

Three DNA Polymerases Cooperate to Back Up the Deficiency of polη in Cells from XPV Patients

Cells from XPV patients exhibit a mild sensitivity to UV and a strong increase in UV mutagenesis, which are thought to cause the high predisposition to sunlight-induced skin cancer characteristic of this disease.[1] This UV hyper-mutability indicates the activity of a backup TLS system, which carries out TLS across CPD with lower efficiency and higher error frequency. The attempts to identify which polymerase performs the bypass in XPV cells focused on polι,[42-45] but did not provide a definitive answer. Adopting the gap-lesion plasmid assay and cellular UV sensitivity assays, along with knockdown of the expression of specific DNA polymerases using siRNA, we were recently able to show that three polymerases are involved in TLS across a TT CPD in XPV cells: polζ, polκ and polι.[46] Moreover, siRNA epistasis analysis revealed a picture generally similar to that observed for the bypass of cisPt-GG and BP-G: knocking-down the expression of *REV3L* caused the biggest effect in reducing the extent of TLS, and a generally similar effect was obtained by knocking-down the expression of *POLK* and *POLI* (encoding polι) combined.[46] Thus, the backup TLS mechanism across a TT CPD in XPV patients consists of two-polymerase reactions, involving polκ, polι and polζ. Possibly polκ and/or polι carry out the insertion step, whereas polζ carries out the extension step (**Fig. 1**). These results provide evidence for the action of two-polymerase mechanisms in a physiologically relevant setting, in which they backup a deficiency caused by a germ-line mutation in a human hereditary disease.

In the bypass of cisPt-GG and BP-G, one inserter polymerase was relatively accurate, whereas another was mutagenic, as described above. In contrast, the mutagenic specificity of TLS across a TT CPD in XPV cells was similar under all conditions examined, regardless of which polymerase was silenced.[46] The simplest explanation for this phenomenon is a dominance of the TT CPD lesion, which dictates the nucleotides inserted, regardless the identity of the DNA polymerase (except for polη, which is of course inactive in these cells).

It should be pointed out that purified recombinant polκ was reported to be unable to bypass a TT CPD,[47,48] nor a cisPt-GG lesion.[47] Our results, on the other hand, suggest a clear involvement of polκ in TLS across these lesions. This discrepancy may be due to a difference between the native polκ and the recombinant purified polκ used in the in vitro studies (e.g., due to a post-translational modification), or due to the lack of accessory proteins[49] which might be needed for the bypass activity of polκ at TT CPD and cisPt-GG adducts.

A Different Role for polκ in TLS Across TT CPD and in Protection of Cells Against UV Cytotoxicity

As mentioned above, polκ and polι seem to back up each other in TLS across a TT CPD in XPV cells, since knocking-down the expression of each had a weak if any effect on TLS, whereas knocking-down the expression of both caused a strong decrease in TLS.[46] However, when cellular UV sensitivity of XPV cells was assayed, knocking-down the expression of *POLK* had a significant effect, whereas knocking-down the expression of *POLI* had none.[46] This indicates, that polκ has an important role in protecting XPV cells against UV damage, consistent with the observation that cells from *Polκ*$^{-/-}$ knockout mice are UV sensitive.[31] Moreover, since UV sensitivity is determined not only by TLS, it suggests that polκ functions also in additional protective processes. XPV cells are NER-proficient, and therefore the polκ effect may be attributed to an involvement in NER.[50] However, an effect of polκ on UV sensitivity was found also in XPA cells,[46] which are NER-deficient, indicating involvement of polκ in protective mechanisms other than TLS or NER. For example, polκ but not polι, may be involved in DNA damage tolerance via homology-dependent repair (homologous recombination or template switch recombination). In addition, it is possible that polκ, but not polι, can act at sites of potentially lethal CPD, such as in overlapping post-replication daughter gaps, or near double-strand breaks,[51,52] where a deficiency in tolerance may cause cell death.

The importance of polκ in providing protection against UV cytotoxicity is illustrated by the surprising finding that in XPA cells, knocking down the expression of *POLK* or *POLH* sensitizes the cells to UV to a similar extent.[46] A possible explanation is that in the absence of NER, UV irradiated XPA cells are overloaded with CPD, polη-promoted TLS is saturated, and as a result the polκ/polζ TLS pathway is activated. This is consistent with the report of an involvement of polζ in replication across CPD in mice cells under a high UV dose,[6] and with the high UV sensitivity of XPA cells in which the expression of *REV3L* was knocked-down.[46] XPA cells are unable to repair CPD, nor 6-4 photoproducts, and therefore in this case UV survival is affected by polζ-mediated TLS across both CPD and 6-4 PP. Indeed, when CPD were repaired by photoreactivation immediately after UV, knocking-down the expression of polη had no effect, as expected, since essentially no CPD remained. Similarly, knocking down the expression of *POLK* had no effect under these conditions, strongly arguing that polk's protection is primarily against CPD. In contrast, knocking down polζ still sensitized cells to UV even after elimination of CPD by photoreactivation, consistent with its role in TLS across 6-4 PP.[46]

Multiple Inserter Polymerases and Fewer Extender Polymerases?

In vitro studies have shown that polκ is an efficient extender.[40] Still, it is polζ that seems to be widely required for TLS. Thus, it appears that the general extender properties of polκ observed in vitro, are not essential for TLS in vivo. Considering TLS across CPD, we prefer the model in which polκ carries out insertion,

whereas polζ carries out extension. Is the opposite model possible too, namely that polζ carries out insertion and polκ extension? Epistasis analysis does not provide a detailed mechanism, and therefore such a possibility cannot be completely ruled out. However, the fact that polι seems to be backing up polκ, argues that the latter acts in the insertion step, since polι is an inserter,[48] and at least in vitro cannot carry out effective extension. The picture that emerges is of multiple inserters, and one main extender, polζ. However, the situation may be more complex, since in addition to the five TLS polymerases, cells have 10 additional DNA polymerases, which function in replication and repair, and may be involved also in TLS. For example, the replicative polymerase polδ was implicated in TLS across an abasic site,[53,54] and polλ in TLS across 8-oxoguanine.[49] In any case, polζ does seem to function as the major extender polymerse. The disadvantage of such a strategy is that polζ becomes a bottleneck in TLS. Potential advantages are: (1) it might be evolutionary easier to evolve a polymerase specialized for accurate insertion only, rather than both accurate insertion and extension; (2) easier regulation of TLS.

So why does polκ exhibit extender properties in vitro? Evidently, the function of polζ is more important than polκ since *POLK* knockout mice grow relatively normally,[31] whereas polζ is essential in mice, as described above. However, it is possible that (1) the extender properties of polκ are a coincidental byproduct of its special structure which evolved to insert opposite an important lesion (BP-G?), or (2) that polκ acts as a specialized extender for specific lesions which were not examined yet.

How is Selection of the "Right" Polymerase for a Specific Lesion Achieved?

TLS is regulated at least at two levels: (1) The access to DNA. Since all TLS polymerases are mutagenic, their access to DNA must be restricted to the site of lesion. (2) The selection of a specific DNA polymerase for a particular damage. A key component in regulating access to DNA is PCNA, and specifically its monoubqiuitination by RAD6-Rad18.[55,56] This is supported by the presence of ubiquitin-binding domains in TLS polymerases,[57] and their tighter binding to monoubiquitinated PCNA.[58,59] We have uncovered an additional layer of regulation of PCNA monoubiquitination, by showing that p53 is required for efficient UV-induced monoubiquitination of PCNA.[60] Similarly, the p53 target gene p21, is also required for efficient monoubiquitination of PCNA.[60]

What happens after PCNA becomes monoubiquitinated? How does the right polymerase reach its target lesion? Is it simple competition between polymerases? The binding affinities of polymerase to their cognate lesions are not sufficient to explain the polymerase selection, however, it is possible that binding affinities are higher and more specific with the monoubiquitinated PCNA. Strikingly, we found that in the absence of p53 the extent of TLS increased, but became less accurate, as if regulation was lifted, and every polymerase gets access to any lesion, the result being higher TLS extent, but at the price of lower fidelity.[60] A similar situation was observed in cells lacking p21.[60] Since under these conditions PCNA ubiquitination is deficient,[60] it might mean that binding affinity to damaged DNA of a specific polymerase increases when it is bound to ubiquitinated PCNA. It would be interesting to measure binding affinties of polκ to BP-G and of polη to cisPt-GG in the presence and absence of ubiquitinated PCNA. In addition, other proteins might be involved in dictating polymerase selection.

It should be noted that in most cell lines used, p53 is mutated or deleted. In fact many of the cell lines used to examine PCNA ubiquitination are SV40-transformed, and therefore p53-deficient, as the large T antigen of SV40 binds and inactivates p53.[61] Thus, PCNA ubiquitination is not totally dependent on p53, and does occur in its absence. However, it appears that in cells in which p53 is not mutated (like U2OS), efficient PCNA ubiquitination requires p53 and p21.[60] It is not yet known whether the effects of p53 and p21 on the extent and accuracy of TLS are mediated only via their effects on ubiquitination of PCNA. Progress in this field is hampered by the fact that on one hand most cell lines, be it immortalized or cancer cells, have defective p53, and on the other hand working with primary cells is very difficult.

Table 1. DNA polymerases involved in two-polymerase TLS reactions across several DNA lesions*

DNA lesion	TLS reaction no.	Insertion	Extension	Outcome
BP-G	1**	Polκ	Polζ	Accurate
	2	Polη	Polζ	Mutagenic
	3	Pol?	Polζ	Accurate?
Overall TLS accuracy: ~90%				
cisPt-GG	1**	Polη	Polζ	Accurate
	2	Polκ	Polζ	Mutagenic
Overall TLS accuracy: ~90%				
TT CPD	1**	Polη	Polη	Accurate
Overall TLS accuracy: ~99%				
	2 (XPV cells)	Polκ	Polζ	Mutagenic
	3 (XPV cells)	Polι	Polζ	Mutagenic
Overall TLS accuracy: ~70%				

*Based on refs. 33 and 46. **These are the main two-polymerase reactions for the indicated DNA lesions.

Concluding Remarks

TLS functions in mammalian cells as an important protective mechanism that enables cells to successfully cope with DNA damage during replication. Recruited from a repertoire of at least 5 DNA polymerases (and perhaps as many as 15) by a regulated process that involves p53, p21 and monoubiquinated PCNA, TLS polymerases bind to sites of DNA lesions located at replication forks and single-strand gaps to carry out the bypass reaction. The mechanism of polymerase selection is unknown yet, but it leads to the relatively accurate bypass of at least some evolutionary important lesions, and this accurate TLS is also fast.[33] Other lesions are bypassed in a mutagenic manner, and those TLS reactions

are much slower than the accurate TLS reactions.[33] TLS is usually carried-out by pairs of DNA polymerases. The first polymerase, an inserter, incorporates a nucleotide opposite the lesion, whereas the second polymerase, an extender, extends the primer terminus created by the inserting polymerase. Several DNA polymerases can act as inserters, such as polη, polκ and polι, whereas extension is carried out primarily by polζ (**Table 1**). More than one two-polymerase reactions can occur at a certain type of lesion, with different mutagenic outcomes. Thus, TLS across a cisPt-GG adduct occurs primarily by a polη-polζ reaction, which is largely accurate, but also by a polκ-polζ reaction, which is more mutagenic (**Table 1**). TLS across a BP-G adduct occurs primarily by a polκ-polζ reaction, which is largely accurate, and to a lesser extent by a more mutagenic polη-polζ reaction. A third pair of polymerases, involving an as yet unknown polymerase along with polζ, is also involved in TLS across BP-G in human cells (**Table 1**). Finally, in cells from XPV patients, who are deficient in polη, TLS across CPD is carried out by reactions involving polκ and polζ, or polι and polζ (**Table 1**). The challenge is to decipher the molecular mechanisms that underlie TLS regulation and polymerase selection, to explain how an inherently mutagenic process mitigates the mutation load caused by DNA damage, and in some cases, provides protection against cancer.

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