

Extra View

Keeping Mammalian Mutation Load in Check

Regulation of the Activity of Error-prone DNA Polymerases by p53 and p21

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p53, p21, translesion DNA synthesis, DNA repair, PCNA, TLS, carcinogenesis

ABBREVIATIONS

BPDE	benzo[a]pyrene diol epoxide
BP-G	benzo[a]pyrene-guanine adduct
cisPt-GG	cisplatin-guanine-guanine adduct
CPD	cyclobutane pyrimidine dimer
PCNA	proliferating cell nuclear antigen
pol	DNA polymerase
TLS	translesion DNA synthesis
MEF	mouse embryonic fibroblasts

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ABSTRACT

To overcome DNA lesions that block replication the cell employs translesion DNA synthesis (TLS) polymerases, a group of low fidelity DNA polymerases that have the capacity to bypass a wide range of DNA lesions. This TLS process is also termed error-prone repair, due to its inherent mutagenic nature. We have recently shown that the tumor suppressor p53 and the cell cycle inhibitor p21 are global regulators of TLS. When these proteins are missing or nonfunctional, TLS gets out of control: its extent increases to very high levels, and its fidelity decreases, causing an overall increase in mutation load. This may be explained by the loss of selectivity in the bypass of specific DNA lesions by their cognate specialized polymerases, such that lesion bypass continues to a maximum, regardless of the price paid in increased mutations. The p53 and p21 proteins are also required for efficient UV light-induced monoubiquitination of PCNA, which is consistent with a model in which this modification of PCNA is necessary but not sufficient for the normal activity of TLS. This regulation suggests that TLS evolved in mammals as a system that balances gain in survival with a tolerable mutational cost, and that disturbing this balance causes a potentially harmful increase in mutations, which might play a role in carcinogenesis.

INTRODUCTION

DNA replication has a remarkable fidelity, estimated to produce 10^{-9} – 10^{-11} mutations/nucleotide, achieved by multiple mechanisms of error avoidance and correction.¹ However, this remarkable fidelity becomes largely irrelevant when replication encounters a DNA lesion, usually a chemically altered base, because the coding information is distorted on one hand, and the lesion may cause a physical barrier to the progression of replication on the other hand. DNA lesions are not rare events. In fact it has been estimated that even without excessive exposure to DNA damaging agents each day genomic DNA suffers no less than 20,000 hits.² These have both intracellular origins, such as spontaneous depurination and oxidation, and external origins, such as sunlight and tobacco smoke. To overcome the potential problems to the functionality of DNA, essentially all organisms use a battery of DNA repair enzymes that identify the damaged site, and remove it with restoration of the original DNA sequence.³

Despite the presence of these error-free DNA repair mechanisms, a significant number of DNA lesions escape repair. During DNA replication these lesions may cause arrest of the replication fork, and/or the formation of replication gaps, which must be processed to complete replication and enable cell division.⁴⁻⁶ One of the central mechanisms to overcome such lesions is translesion DNA synthesis (TLS), also termed translesion replication (TLR), or error-prone repair. The key components in this process are specialized DNA polymerases, characterized by a low fidelity and the ability to replicate across DNA lesions, even bulky ones. Because coding information of the modified bases is usually distorted, TLS is inherently an error-prone process, hence the term error-prone repair: the gap is repaired, but a mutation is produced.⁷⁻⁹

REGULATED ACTION OF THE SPECIALIZED POLYMERASES ENSURES THAT SOME DNA LESIONS ARE BYPASSED RELATIVELY EFFICIENTLY AND ACCURATELY, BUT OTHERS ARE NOT

The study of TLS in mammals lead to two surprises: (1) The discovery of many specialized, low-fidelity, DNA polymerases that are capable of bypassing DNA lesions

(10 altogether; but not all have been proven to function in TLS).¹⁰ This seems counterintuitive given the potential mutagenic nature of these polymerases and the role of mutations in cancer. (2) TLS may protect humans against cancer, at least in some cases, despite its inherent mutagenic nature. This notion is based on the hereditary disease xeroderma pigmentosum variant (XPV), which is characterized by extreme sunlight sensitivity and high predisposition to skin cancer, caused by a defective specialized DNA polymerase, pol η .^{11,12} The explanation proposed for this phenotype is that pol η is specialized for TLS across the main sunlight DNA damage, cyclobutane pyrimidine dimers (CPD), such that it performs the bypass more accurately and more efficiently than any other polymerase in the cell. When pol η is defective another polymerase bypasses the CPD, but does it in a less efficient and more mutagenic manner. What this suggests is that under normal conditions pol η , a low-fidelity polymerase, protects humans against cancer. Is this a general characteristic of the entire TLS system, or is it unique to pol η and CPD? In other words, has the multiplicity of specialized and mutagenic polymerases in mammals evolved to endow replication with the ability not only to bypass lesions, but also to do it in a manner that will minimize the mutational load of this process? To date there are three examples of TLS reactions in which a specialized DNA polymerase bypasses its cognate DNA lesion with higher efficiency and higher fidelity than any other polymerase in the cell: pol η and the UV light-induced CPD,^{11,12} as mentioned above, pol κ and benzo[*a*]pyrene-guanine (BP-G),^{13,14} a major tobacco smoke-induced DNA lesion, and pol η and cisplatin-GG,¹⁵ an adduct produced by a drug used in cancer chemotherapy. Is this true for all types of DNA damage? The answer is most likely negative because of the simple reason that the number of types of DNA lesions far exceeds the number of specialized DNA polymerases. Indeed, there are cases in which the bypass is carried out with an accompanied high mutagenicity, e.g., TLS across UV lesions (other than CPD) by REV3,¹⁶ the catalytic subunit of the putative pol ζ . In that case, it appears that to be able to bypass, the price is an accompanied high mutability. This suggests that the TLS system functions with first priority to bypass replication barriers, but in certain cases, specific polymerases evolved which achieve the best of the two worlds: High bypass efficiency, and relatively good fidelity.

REGULATION OF TLS IS CRITICAL TO AVOID AN ESCALATION IN MUTATION LOAD

The notion that when a particular DNA polymerase is absent, another polymerase performs the bypass, at times with lower efficiency and fidelity, highlights the potential threat to the cell posed by the multiplicity of mutagenic TLS polymerases. Obviously, regulation of TLS may be employed as a means to prevent an escalation in the mutational load and control cancer risk. Such regulation must ensure that (1) the specialized polymerases act only when needed, and (2) that polymerases act only at the right location in DNA, e.g., at their cognate lesions. One of the components in this regulation is the binding affinity to, and bypass ability of a particular DNA lesion by its cognate specialized DNA polymerase. Indeed, some polymerases act on a damaged template with higher catalytic efficiency than on undamaged template, but at least in vitro the discrimination is usually weak.^{17,18} In addition, although purified DNA polymerases do show preferred bypass of specific types of lesions, there are significant overlaps in lesions bypassed by a particular polymerase.³ Taken together this would suggest that there must exist additional regulatory mechanisms for TLS. In contrast to the *E. coli* specialized polymerases,

mammalian TLS polymerases are constitutively expressed, showing a wide tissue distribution.³ This does not mean that their expression is not regulated at the transcriptional level, as indicated by the inducibility of pol κ at the transcriptional level by polycyclic aromatic hydrocarbons.¹⁹ However, it does seem based on current data that this mode of regulation has secondary importance. One clear example is the observation that the expression of pol η is not induced by UV light;²⁰ but it is induced by DNA breaks.²¹ The constitutive expression of TLS polymerases suggests that regulation will be primarily at the post-translational level.

UBIQUITINATION OF PCNA, THE SLIDING DNA CLAMP, REGULATES TLS

A key element in regulating TLS discovered in the yeast *S. cerevisiae* is the monoubiquitination of PCNA.^{22,23} PCNA is the universal DNA sliding clamp, which serves as the processivity factor of the replicative pol δ (and pol ϵ). PCNA interacts with many other proteins involved in responses to DNA damage, illustrating its key role in these processes.²⁴ Moreover, PCNA was shown to interact with several specialized DNA polymerases.⁸ While usually ubiquitination involves the modification of proteins by multiple ubiquitin residues and functions to promote the proteolysis of proteins,²⁵ the monoubiquitination of PCNA at Lys164 signals a change in functional specificity. In *S. cerevisiae* it was shown that this modification of PCNA switches the normal high-fidelity DNA replication into an error-prone mode, in which the specialized polymerases participate.²³ The enzymes responsible for this monoubiquitination are RAD6, which is an E2 ubiquitin-conjugating protein, and RAD18, which is an E3 ubiquitin ligase.⁸ Two recent in vitro studies on the effect of PCNA ubiquitination on DNA polymerases purified from *S. cerevisiae* yielded conflicting results, leaving this problem still open. One study reported that ubiquitinated PCNA stimulates bypass across abasic site by pol η and REV1,²⁶ whereas another study reported no effect of the modified PCNA on pol δ , η , ζ and REV1.²⁷ PCNA also undergoes polyubiquitination at the same position, which depends on Mms2, Ubc13 and Rad5 proteins, and in addition it undergoes SUMOylation.^{22,23}

In mammals PCNA is monoubiquitinated at the same Lys164 after treatments with several DNA damaging agents such as UV, cisplatin and benzo[*a*]pyrene diol epoxide (BPDE),²⁸⁻³¹ but its involvement in TLS has not been demonstrated yet. It was reported, however, that pol η and pol ι selectively bind monoubiquitinated PCNA.^{28,32} Similarly, in BPDE-treated human cells pol κ was found to selectively bind monoubiquitinated PCNA.³⁰ A plausible, but yet unproven, model is that the PCNA is monoubiquitinated when the replicative polymerase stalls at the blocking lesion, and this serves as an anchor for targeting specialized polymerases specifically to the location of the DNA damage. The presence of a ubiquitin-binding domain in the specialized DNA polymerases of the Y family supports such a model.³² Recently it was reported that PCNA monoubiquitination is subject to regulation by a deubiquitinating enzyme (DUB) termed USP1. Upon UV irradiation USP1 is inactivated through an autocleavage event, thereby enabling accumulation of monoubiquitinated PCNA.³³

THE P53 AND P21 PROTEINS SUPPRESS THE EXTENT OF TLS, BUT MAKE IT MORE ACCURATE

We have recently shown that two key proteins of the DNA damage response and cell cycle regulation, p53 and p21, are master

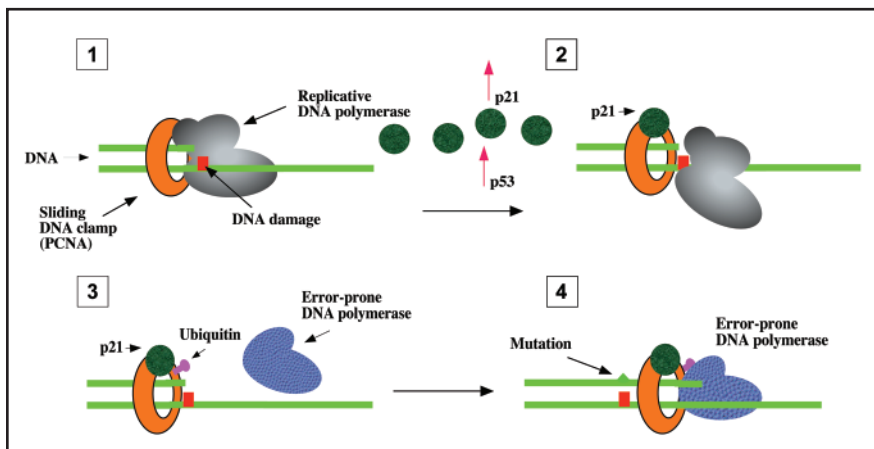


Figure 1. A model for the regulation of translesion DNA synthesis by p53 and p21. Inhibition of replication at a DNA damage leads to the activation of p53, which then induces p21. The p21 protein binds PCNA, thereby causing dissociation of the replicative DNA polymerase δ . This increases accessibility of the ubiquitination site in PCNA (lys164) and the DNA damage, leading to facilitated monoubiquitination of PCNA, binding of the specialized polymerase, and lesion bypass. See text for details.

regulators of TLS, acting to suppress the efficiency of TLS, but increase its accuracy.³⁴ The p53 protein is a major tumor suppressor, which is mutated in approximately 50% of all human cancers. It exerts its effects on error-free DNA repair, cell cycle arrest and apoptosis primarily via its activity as a transcription factor that controls over a hundred genes.^{35,36} Notably, one of the genes regulated by p53 is p21, a cell cycle inhibitor that acts by inhibiting cyclin-dependent kinases.³⁷ Remarkably, p21 was also shown to bind PCNA³⁸ at a site overlapping the binding site of pol δ ,³⁹ and located not far away from the ubiquitination site of PCNA. In our study we have utilized a quantitative TLS model assay system based on transfection of cultured cells with a gapped plasmid carrying a site-specific damaged nucleotide at the gap region, along with a control gapped plasmid without a damaged nucleotide. The plasmids do not replicate in the mammalian cells, enabling the measurement of the extent and misinsertion specificity of TLS-mediated gap filling by extracting the plasmids from the mammalian cells and using them to transform an indicator *E. coli* strain (which is defective in TLS). Although this assay system measures TLS outside the contexts of the chromosome and replication, it has the advantage that it is quantitative.^{14,40,41} In fact it is currently the only quantitative TLS assay available for mammalian cells. Three types of DNA lesions were used in the study, representing TLS by different polymerases: BP-G, bypassed primarily by pol κ , cisplatin-GG, bypassed primarily by pol η , and a synthetic abasic site, bypassed by an aphidicolin-sensitive polymerase, perhaps pol δ . Somewhat surprisingly, p53 was found to suppress the efficiency of TLS across each of the three lesions. When the fidelity of TLS was measured, it was found that in the presence of p53, bypass across BP-G and cisPt-GG was twofold more accurate than without p53. This was found both in human H1299 cells carrying a temperature sensitive p53 gene, as well as in p53^{-/-} mouse embryonic fibroblasts (MEF). The overall effect was a 7-10 fold decrease in the actual number of mutants obtained.³⁴ A similar effect was obtained when MEF from p21^{+/+} and p21^{-/-} cells were compared, suggesting that the p53 effect is mediated primarily via its ability to induce p21, and that it is p21 that affects TLS. The effect of p21 was mediated via its PCNA-binding domain, arguing for a direct effect of p21 on the TLS machinery.³⁴

As those results were obtained with a nonreplicating, plasmid-based assay system, the question arises whether the p53 and p21 regulation is relevant to chromosomal TLS. There are currently no methods for directly assaying chromosomal TLS. It is possible, however, to assay its manifestation as mutagen-induced chromosomal mutagenesis. Using such assays it was previously reported that p53 suppresses chromosomal UV mutagenesis, consistent with the effect of p53 on TLS.^{42,43} However, since p53 is known to be required for efficient excision repair,⁴⁴ its effect on mutagenesis may be indirect, namely by reducing the number of DNA lesions on which TLS acts. This question was addressed in another study that compared UV mutagenesis in cells with or without p53. It was found, again, that p53 suppressed UV mutagenesis, without affecting excision repair, as assayed by unscheduled DNA synthesis.⁴⁵ The authors suggested that p53 suppresses UV mutagenesis by a yet unknown pathway, which seems now to be the regulation of TLS.

The power of a model assay system such as the gapped plasmid TLS assay is that it provides a basis for further studies in the more complex chromosomal context. One mechanism by which p53 and p21 could exert their effect on TLS is via monoubiquitination of PCNA, and this could be assayed in the chromosomal context. It should be emphasized that p53 and p21 were not expected to be essential for the monoubiquitination of PCNA, since it occurs in SV40-transformed cell lines, where p53 is inactivated.²⁸ Moreover, we have observed UV-induced monoubiquitination also in H1299 cells, which are p53-null.³⁴ However, we did find that both p53 and p21 were required for efficient UV-induced monoubiquitination of PCNA. This was shown by the temperature-sensitive p53Val135 mutant, and by knocking-down p53 and p21 expression using p53 shRNA and p21 siRNA, respectively.³⁴

MODEL FOR THE REGULATION OF TLS BY P53 AND P21

Based on the known properties of p53 and p21, we propose the following working model for their mechanism of action in regulating TLS (Fig. 1). The arrest of replication at a blocking DNA lesion activates p53, which in turn induces p21. The p21 protein binds PCNA, thereby causing release of pol δ from the damaged DNA site and from the PCNA. Since pol δ is a bulky enzyme whereas p21 is small, the net effect of the displacement of pol δ by p21 is an increased accessibility both to the damaged DNA site and to the PCNA molecule located in its vicinity. This clearing of the 'replication arrest' zone may fulfill at least two functions: (a) it increases the access of the ubiquitination enzymes to Lys164, which is located near the pol δ binding site on PCNA, thereby facilitating the monoubiquitination of PCNA; (b) it frees the way for the access of the specialized DNA polymerases to the damaged site. Once PCNA is monoubiquitinated, it triggers the binding of the specialized polymerases. The simplest possible mechanism for the effect of PCNA monoubiquitination is the direct binding of the specialized polymerases to the monoubiquitinated site on PCNA. This is supported by the finding of a ubiquitin-binding domain in several TLS polymerases,³² and by the demonstration of selective binding of pol η ,^{28,32} pol ι ³² and pol κ ³⁰ to monoubiquitinated PCNA. However, two indirect modes

of binding may also be involved: binding via an adaptor protein(s), and binding of the specialized polymerase after additional cellular events triggered by the monoubiquitination of PCNA. These models are not mutually exclusive.

Although, according to this model, the role of p53 is to induce p21, p53 might have additional roles in regulating TLS. Expressing p21 from a vector in the human cell line H1299, which is p53 null, or in p53^{-/-} mouse embryonic fibroblasts led to only 50% complementation of the defect in regulation of TLS.³⁴ This may result from a technical difficulty in the complementation, but it may mean that there is an additional function for p53 in TLS. Such a putative function may include an additional p53-regulated protein, or even the direct action of p53, which has a ssDNA-binding domain distinct from its dsDNA-binding domain,^{46,47} and possesses a 3' → 5' exonuclease activity.⁴⁸

It was recently reported that ectopic expression of p21 in stably transfected cells inhibited UV-induced monoubiquitination of PCNA. Unlike in our case, where the effect of p21 on TLS was mediated via its PCNA-interacting domain, this effect was mediated via the CDK-binding domain of p21.⁴⁹ Since that study did not include siRNA knock-down of p21, it is not directly comparable to our study. However, the regulatory activities of p21 are complex, and involve DNA repair activities other than TLS.⁵⁰ Therefore, additional studies are needed to fully elucidate the physiologically relevant effects of p21 on TLS.

THE REGULATION OF TLS BY P53 AND P21 INVOLVES ELEMENTS OTHER THAN PCNA MONOUBIQUITINATION

In cells in which p53 or p21 is missing or nonfunctional, TLS seems to get out of control. Its extent significantly increases, and its fidelity decreases, causing an overall increase in mutations. This may be explained by the loss of the selectivity in the bypass of specific DNA lesions by their cognate specialized polymerases. Thus, in the absence of the 'guardians', any polymerase gets access to the damaged site in DNA, and a chance to perform bypass. Multiple attempts by many polymerases occur, dictated most likely by the local concentrations of the polymerases, their affinity to the damaged site in DNA, and their inherent ability to bypass the lesion. How do p53 and p21 ensure the 'well-behaved' balanced action of TLS with tolerable mutagenicity? The regulation of PCNA monoubiquitination by p53 and p21 is only part of the answer, and there must be an additional important mechanistic determinant, simply because monoubiquitination occurs also when p53 is inactivated. Thus, monoubiquitination of PCNA might be necessary, but not sufficient for the full regulatory activity of p53 and p21 in TLS. It is not yet clear what additional mechanisms underlie the regulation of TLS by p53 and p21.

The p53 protein was termed the 'Guardian of the Genome',⁵¹ and its role in regulating the mutation load caused by TLS might be a molecular manifestation of this function. It is tempting to speculate that the increased mutagenicity caused by TLS in a cell in which p53 is mutated, facilitates its progression along the path of carcinogenesis. This may be one of the reasons for the critical role of p53 as a tumor suppressor.

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