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Eukaryotic translesion synthesis: choosing the right tool for the job

Kyle T. Powers and M. Todd Washington*

Department of Biochemistry, University of Iowa College of Medicine, Iowa City, IA 52242-1109

Abstract

Normal DNA replication is blocked by DNA damage in the template strand. Translesion synthesis is a major pathway for overcoming these replication blocks. In this process, multiple non-classical DNA polymerases are thought to form a complex at the stalled replication fork that we refer to as the mutasome. This hypothetical multi-protein complex is structurally organized by the replication accessory factor PCNA and the non-classical polymerase Rev1. One of the non-classical polymerases within this complex then catalyzes replication through the damage. Each nonclassical polymerase has one or more cognate lesions, which the enzyme bypasses with high accuracy and efficiency. Thus, the accuracy and efficiency of translesion synthesis depends on which non-classical polymerase is chosen to bypass the damage. In this review article, we discuss how the most appropriate polymerase is chosen. In so doing, we examine the structural motifs that mediate the protein interactions in the mutasome; the multiple architectures that the mutasome can adopt, such as PCNA tool belts and Rev1 bridges; the intrinsically disordered regions that tether the polymerases to PCNA and to one another; and the kinetic selection model in which the most appropriate polymerase is chosen via a competition among the multiple polymerases within the mutasome.

Keywords

DNA polymerase; DNA repair; DNA replication; Genome instability; Mutasome; PCNA; Protein-protein interactions; Structure

1. Introduction

DNA damage in the template strand blocks DNA synthesis by classical DNA polymerases – those involved in normal DNA replication and repair. Consequently, when replication forks encounter DNA damage, they stall. Without a means of replicating through damaged DNA, genome instability, chromosome instability, and cell death can occur. Thus, several damage

*To whom correspondence should be addressed: M. Todd Washington, Department of Biochemistry, 4-403 Bowen Science Building, University of Iowa, Iowa City, IA 52242-1109. Phone: 319-335-7518, Fax: 319-335-9570, toddwashington@uiowa.edu.

Conflict of interest

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bypass pathways have evolved that allow replication to proceed through damaged DNA. Translesion synthesis (TLS) is a major damage bypass pathway in eukaryotes. In TLS, one or more non-classical DNA polymerases – those involved in damaged DNA replication – catalyze DNA synthesis through the DNA lesion (1–11). Unfortunately, the bypass of DNA damage by nonclassical DNA polymerases is often error-prone and mutagenic.

Cells possess a variety of non-classical polymerases for replicating through DNA damage. These non-classical polymerases differ from classical DNA polymerases in that they can utilize DNA lesions as templates for nucleotide incorporation. Each of these polymerases has one or more DNA lesions that it bypasses with relatively high accuracy and efficiency as determined by steady state or pre-steady state kinetics (for a recent discussion of this topic, see (12)). These lesions are known as the cognate lesions of the non-classical polymerases (Table 1). For example, the cognate lesions of DNA polymerase eta (**pol η**) include thymine dimers and 8oxoguanines (13–15). The cognate lesions of Rev1 include minor-groove and exocyclic guanine adducts (16,17).

The accuracy and efficiency of TLS depends on which of the available non-classical polymerases is chosen to bypass the DNA damage. If the damage is a cognate lesion for the chosen polymerase, the accuracy and efficiency of TLS will be relatively high. If the damage is not a cognate lesion for the polymerase, the accuracy and efficiency will be much lower. Thus, the central question in trying to understand the accuracy and efficiency of TLS is: how is a non-classical polymerase chosen to bypass DNA damage? Answering this question will involve investigating the structures and functions of the individual proteins involved in TLS as well as the structures and functions of any protein complexes that they form.

These non-classical polymerases interact with each other and with several other replication-associated proteins. This has led to the general working hypothesis that these polymerases function within a large, multi-protein complex that forms at stalled replication forks (18–20). In this article, we are adopting this theoretical framework and are using the term “mutasome” (*i.e.*, mutagenic replisome) to refer to this complex. These multiple non-classical polymerases contain intrinsically disordered regions that act as flexible tethers allowing the polymerases to sample a wide range of conformational states without dissociating from the mutasome (21,22). This conformational flexibility presumably allows the multiple polymerases within the mutasome to compete with one another for binding the DNA substrate. Ultimately, such a competition would maximize the accuracy and efficiency of TLS via a kinetic selection model (12).

In this review article, we will examine this emerging paradigm. Following in the footsteps of Aristotle, we will first discuss the composition of the mutasome – its material cause. This will include an examination of the intrinsically disordered regions of the non-classical polymerases and the structural motifs that mediate the protein-protein interactions within the mutasome. We will next discuss the structural organization of the mutasome – its formal cause. This will include an examination of both the architectures and high-resolution structures that the mutasome can adopt. We will then discuss the assembly of the mutasome – its efficient cause. This will include an examination of the potential roles that PCNA ubiquitylation plays in TLS. We will lastly discuss how the mutasome functions to maximize

the accuracy and efficiency of TLS – its final cause. This will include an examination of the kinetic selection model for choosing the most appropriate nonclassical polymerase to bypass the lesion.

2. Material cause: What are the components of the mutasome?

The components of the mutasome are highly conserved among eukaryotes. All of the yeast proteins involved in TLS have clear orthologs in mammalian systems, although mammalian systems possess additional paralogs of some of the yeast proteins. Moreover, all of the protein-protein interaction motifs found in the yeast system are present in mammalian systems, although mammalian systems contain more copies of these motifs. Because of this added complexity of mammalian systems, the yeast system lends itself more readily to addressing fundamental structural and mechanistic questions. For this reason, we will focus primarily on the yeast system.

2.1. PCNA

The central component of the mutasome is proliferating cell nuclear antigen (**PCNA**) – a key replication accessory factor and remnant of the stalled replication fork (23–27). PCNA is a ring-shaped protein comprised of three identical subunits. PCNA encircles double-stranded DNA and acts as a sliding clamp. The PCNA ring has a front face oriented toward the direction of DNA synthesis and a back face oriented away from the direction of DNA synthesis. PCNA interacts with a wide range of proteins involved in DNA replication and repair. Many of these interacting proteins contain short, sequence motifs known as PCNA-interacting protein (**PIP**) motifs (23,28–33). These motifs are often located in intrinsically disordered regions of these interacting proteins and usually contain two adjacent aromatic residues (phenylalanine or tyrosine residues). The aromatic residues of PIP motifs bind in a hydrophobic pocket on the front face of the PCNA ring.

2.2. Rad6-Rad18

Rad6-Rad18 is a ubiquitin-conjugating enzyme and ubiquitin ligase complex, which catalyzes the mono-ubiquitylation of PCNA during TLS (34–38). The stoichiometry of this complex is unclear. The X-ray crystal structure of Rad6 shows that it is fully folded (39). By contrast, Rad18 is mostly intrinsically disordered and contains several small, folded domains including a RING domain (residues 28–65), an ubiquitin-binding, zinc-binding (**UBZ**) motif (residues 188–210), a SAP domain (residues 278–312), and a Rad6-binding domain (residues 371–410) (40,41). Rad6-Rad18 interacts with PCNA, Rev1, and pol η (42,43), although the structural bases of these interactions are currently unknown. Furthermore, although the monoubiquitylation of PCNA is likely important directly or indirectly for assembling the mutasome, it is unclear whether this protein remains part of the mutasome after catalyzing PCNA ubiquitylation.

2.3. Rev1

Rev1 is a monomeric non-classical polymerase that plays an important role in structurally organizing the mutasome (11,44,45). Steady state kinetics shows that the Rev1 accurately and efficiently incorporates nucleotides opposite minor-groove and exocyclic guanine

adducts, which are caused by exposure to various chemicals including alkylating agents (16,17). The X-ray crystal structure of the polymerase domain of Rev1 shows that this enzyme uses an unusual template-directed mechanism of DNA synthesis whereby an active site arginine residue serves as the template for nucleotide incorporation (46–48). Because Rev1 only efficiently incorporates nucleotides opposite damaged and non-damaged guanines, it generally cannot extend the primer strand beyond the template lesion. Thus, Rev1 functions as an “insertor” during TLS and requires the assistance of an “extender” polymerase – usually DNA polymerase zeta (**pol ζ**) (10,49).

Rev1 has a polymerase domain (residues 305–738) and two intrinsically disordered regions: one on the N-terminus (residues 1–304) and one on the C-terminus (residues 739–985) (7,12,21). The N-terminal disordered region contains a BRCT domain (residues 163–250). This small domain binds to the side of the PCNA ring at a site that partially overlaps with the PIP motif-binding site on the front of the PCNA ring (Fig. 1) (50,51). The C-terminal disordered region contains two small ubiquitin-binding motifs (**UBM**) (residues 751–779 and 809–837), which are believed to interact with the ubiquitin moiety on ubiquitin-modified PCNA (52). The C-terminal region also contains a small C-terminal domain (**CTD**) (residues 875–985) (53–55). This four-helix bundle binds to a region of the catalytic core of DNA polymerase zeta (**pol ζ**) (Fig. 1) (18,19). It also binds to the PIP motif of pol η in a hydrophobic cleft on the CTD at a distinct site from where pol ζ binds (Fig. 1) (56). In fact, these structural studies directly show that the CTD of Rev1 can bind pol ζ and pol η simultaneously.

2.4. DNA polymerase ζ

Pol ζ is a tetrameric non-classical polymerase comprised of the Rev3 catalytic subunit and the Rev7, Pol31, and Pol32 accessory subunits (57–61). Steady state kinetics shows that pol ζ does not efficiently catalyze the incorporation of nucleotides opposite DNA lesions, but it does efficiently catalyze extension from primerterminal base pairs containing a wide range of template DNA lesions (10,49,62). Thus, pol ζ likely functions as an “extender” during TLS and requires the assistance of an “insertor” polymerase – usually Rev1. There is currently no X-ray crystal structure of the polymerase domain pol ζ, but disorder prediction algorithms show that the Rev3 catalytic subunit, the Rev7 accessory subunit, and the Pol31 accessory subunit are mostly structured. The Pol32 accessory subunit, by contrast, has an intrinsically disordered region on the C-terminus (residues 123–350). This disordered region contains a PIP motif (residues 338–345) that presumably binds PCNA (Fig. 1).

2.5. DNA polymerase η

Pol η is a monomeric non-classical polymerase (13). Steady state and pre-steady state kinetics show that pol ζ accurately and efficiently incorporates nucleotides opposite thymine dimers, which are caused by exposure to ultraviolet radiation, and 8-oxoguanines, which are caused by exposure to reactive oxygen species (13–15). It also efficiently catalyzes extension from primer-terminal base pairs containing these DNA lesions. Thus, pol ζ functions as both an “insertor” and an “extender” during TLS. The X-ray crystal structure of the polymerase domain (residues 1–512) of pol η shows that this enzyme has a larger active site than those of classical DNA polymerases and this allows both template bases of the

thymine dimer to fit into the enzyme's active site without steric clashes (63–66). Pol η has one intrinsically disordered region on the C-terminus (residues 513–632) (7,12,21). This disordered region contains a small UBZ motif (residues 549–582), which is believed to interact with the ubiquitin moiety on ubiquitin-modified PCNA (52). This disordered region also contains a PIP motif (residues 621–628), which binds to PCNA or to the CTD of Rev1 in a mutually exclusive manner (Fig. 1) (20,56).

3. Formal cause: How is the mutasome structurally organized?

Because of their ability to interact simultaneously with multiple binding partners, PCNA and Rev1 play important roles in structurally organizing the mutasome. Thus, it is not surprising that studies of the structural organization of the mutasome have so far focused on PCNA, Rev1, and pol η – the best studied of the non-classical polymerases. The structural organization of the mutasome is being examined on two levels. The first level is the architecture of the complex – *i.e.*, which proteins interact with which other proteins and what structural motifs mediate these interactions. The second level is the high-resolution structure of the complex and includes the range of conformational states that the complex can adopt.

3.1. PCNA tool belts and Rev1 polymerase bridges

Given the types of protein-protein interaction motifs described above, there are two types of architecture possible for the core components of the mutasome. One such architecture is the PCNA tool belt. In this case, Rev1 binds to one subunit of the PCNA ring via its BRCT domain and pol η binds to another subunit of PCNA via its PIP motif. In a PCNA tool belt, PCNA directly binds to both Rev1 and pol η , but pol η and Rev1 do not directly interact with each other. This is because the PIP motif of pol η is bound to PCNA and is not available for binding Rev1. The second architecture is the Rev1 polymerase bridge. In this case, Rev1 and PCNA interact via the Rev1 BRCT domain and Rev1 and pol η interact via the Rev1 CTD and the pol η PIP motif. In a Rev1 polymerase bridge, Rev1 directly binds PCNA and pol η , but pol η and PCNA do not directly interact. This is because the PIP motif of pol η is bound to the Rev1 CTD and is not available for binding PCNA.

Recently, single-molecule total internal reflection fluorescence (**TIRF**) microscopy experiments were performed using purified proteins to determine whether PCNA tool belts and Rev1 polymerase bridges can form among these three proteins (20). Pol η was immobilized on the surface of the microscope slide. PCNA and Rev1 were labeled with different fluorophores and added to the microscope chamber. Ternary complexes were observed in which PCNA and Rev1 simultaneously bound to the immobilized pol η . Two classes of ternary complexes were observed: those in which PCNA bound first followed by Rev1 and those in which Rev1 bound first followed by PCNA. The PCNA-first complexes were PCNA tool belts, because when PCNA binds first to pol η , it does so via PIP motif of pol η . The Rev1 that binds next cannot bind the pol η PIP motif, and thus must bind PCNA via its BRCT domain. Likewise, the Rev1 first complexes were Rev1 polymerase bridges, because when Rev1 first binds pol η , it does so via the Rev1 CTD and the pol η PIP motif. The PCNA that binds next cannot bind the pol η PIP motif, and thus must bind Rev1 via its

BRCT. This study showed that 60% of the ternary complexes formed in this assay were PCNA tool belts and 40% were Rev1 polymerase bridges.

The interpretation of the PCNA-first events as PCNA tool belts and the Rev1-first ternary complexes as Rev1 polymerase bridges was further supported by the use of mutant proteins in the single-molecule TIRF assay. The clearest evidence came from studies in which the CTD of Rev1 was mutated so that it could not interact with pol η . This Rev1 mutant protein should form PCNA tool belts normally, but should be impaired in its ability to form Rev1 polymerase bridges. When studies were done using this mutant Rev1 protein, the number of PCNA tool belts was unchanged but a 10-fold reduction in the number of Rev1 polymerase bridges was observed. This provided compelling evidence that these three proteins form PCNA tool belts and Rev1 polymerase bridges with approximately equal frequencies.

3.2. Flexible tethers

X-ray crystallography has revealed the structures of the polymerase domains of Rev1 and pol η at high resolution (46,63). In addition to their polymerase domains, both of these proteins have large, intrinsically disordered regions that are not amenable to X-ray crystallography (7,12,21). Thus, other structural approaches are necessary to study the high-resolution structures of full-length Rev1 and pol η as well as the range of conformational states that these proteins can adopt. This is the first step toward understanding the high-resolution structures and conformational flexibility of the different architectures of the mutasome.

Recently, a combination of Brownian dynamics (BD) simulations and small-angle X-ray scattering (SAXS) measurements were carried out to examine the high-resolution structure and conformational flexibility of full-length pol η (22). BD simulations were carried out for three builds of full-length pol η : one in which the C-terminal region was entirely unstructured, one in which the C-terminal region was unstructured except for the UBZ motif, and one in which the C-terminal region was unstructured except for the UBZ motif and three putative α -helices obtained from on-line secondary structure prediction methods. The best agreement between the experimental SAXS data and the BD simulations was obtained from the build of full-length pol η containing the structured UBZ and the putative α -helices. This experimentally validated BD simulation shows that the C-terminal region of pol η has high conformational flexibility and can adopt a wide range of conformational states.

The high conformational flexibility of the C-terminal region of pol η and the fact that its PIP motif is located within this region suggests that the C-terminal region of pol η acts as a tether linking the pol η polymerase domain to PCNA (Fig. 2). In addition, BD simulations described below suggest that the intrinsically disordered N-terminal region of Rev1 also act as a flexible tether linking the Rev1 polymerase domain to PCNA (Fig. 2). The tethers in these proteins allow the multiple polymerase domains found within the mutasome to sample a wide range of conformational states without dissociating from the mutasome or changing its architecture. This conformational flexibility allows the multiple polymerase domains to compete with one another for binding the DNA substrate. This competition for binding the

DNA substrate is a critical feature in selecting of the most appropriate non-classical polymerase to carry out DNA damage bypass (see below).

4. Efficient cause: How is the mutasome assembled?

When the replisome encounters DNA damage, it stalls and disassembles. Little is known about the disassembly of the replisome. What is clear is that PCNA is left behind and becomes the nucleation point of the nascent mutasome. Here we will consider the order of assembly of the mutasome and the role that PCNA monoubiquitylation may play in mutasome assembly.

4.1. Order of assembly

UV survival studies in yeast have revealed the genetic relationships between the genes encoding Rev1, pol ζ , and pol η (67,68). The gene encoding Rev1 is epistatic with the genes encoding pol ζ , but not with the gene encoding pol η . Likewise, the genes encoding pol ζ are epistatic with the gene encoding Rev1, but not the gene encoding pol η . By contrast, the gene encoding pol η is epistatic with neither the gene encoding Rev1 nor the genes encoding pol ζ . These studies show that Rev1 and pol ζ function together in bypassing UV-induced DNA lesions, while pol η functions independently. Moreover, the bypass of UV-induced DNA lesions by Rev1 and pol ζ is accompanied by a dramatic increase in the frequency of mutations, whereas the bypass of these lesions by pol η is not.

Studies in human cells have revealed the order of recruitment of these non-classical polymerases to stalled replication forks (69–71). Upon exposure to UV radiation, Rev1, pol ζ , and pol η co-localize with PCNA at replication foci. The localization of Rev1 to replication foci depends on neither pol ζ nor pol η . The localization of pol ζ depends on Rev1, but not on pol η . The localization of pol η depends on neither Rev1 nor pol ζ . These studies show that Rev1 and pol η are recruited independently of one another and independently of pol ζ . However, pol ζ recruitment requires Rev1, but not pol η . These findings support a model in which Rev1 and pol η can be recruited to stalled replication forks via direct interactions with PCNA in either order. Pol ζ , however, is recruited to stalled replication forks via direct interactions with Rev1 at the same time as or subsequent to Rev1 recruitment.

4.2. PCNA ubiquitylation

PCNA mono-ubiquitylation by Rad6-Rad18 is an essential step in TLS. Despite its clear importance, the role of PCNA ubiquitylation in TLS remains unknown. The prevailing view is that PCNA ubiquitylation is a signal to recruit non-classical polymerases (52). Both Rev1 and pol η have ubiquitin-binding motifs in their Cterminal intrinsically unstructured regions. Furthermore, the activities of both Rev1 and pol η are enhanced to a greater extent by binding ubiquitin-modified PCNA versus unmodified PCNA (72). This has been taken as support of the notion that interactions between the ubiquitin-moiety on ubiquitin-modified PCNA and the ubiquitin-binding motifs of Rev1 and pol η act to recruit these non-classical polymerases to stalled replication forks and regulate their functions.

Several observations, however, challenge the prevailing view. First, human pol η interacts with Rad18, and this interaction has been suggested to play a role in recruiting pol η to stalled replication forks and vice versa (43,73). Second, the presence of pol η increases the PCNA ubiquitylation activity of Rad6-Rad18 *in vitro* (74,75). This implies that pol η is already present in the complex with Rad6-Rad18 and PCNA before the ubiquitin is transferred from Rad6 to PCNA.

These findings raise the possibility that it is not the ubiquitylation of PCNA *per se* that recruits non-classical polymerases to stalled replication forks. It may instead be the ubiquitylation machinery itself that recruits these polymerases. In such a model, Rad6-Rad18 is directed to stalled replication forks possibly via interactions with replication protein A (RPA) (76). Pol η is then recruited to the stalled fork either by riding “piggy back” on Rad6-Rad18 as it is directed to the stalled forks or by interacting with Rad6-Rad18 after it has been recruited to the stalled fork. The ubiquitin is transferred to from Rad6 to PCNA once Rad6-Rad18 and pol η are both present at the stalled fork.

5. Final cause: How is the right non-classical polymerase selected?

The accuracy and efficiency of TLS depends on which of the non-classical polymerases in the mutasome is chosen to bypass the DNA damage. The accuracy and efficiency of TLS will be relatively high, if the damage is a cognate lesion for the chosen polymerase. Otherwise, the accuracy and efficiency will be much lower. In this section, we will examine how non-classical polymerases are chosen to bypass DNA damage.

5.1. Ordered recruitment

A major factor that could potentially affect which non-classical polymerase is chosen to bypass the DNA damage is the order of non-classical polymerase recruitment. For example, if pol η is recruited prior to the other non-classical polymerases, it will be the only polymerase in the mutasome prior to the recruitment of Rev1 and pol ζ . During this phase of mutasome assembly, pol η will be able to attempt damage bypass without competition from other non-classical polymerases. It would make sense for pol η to act as a “first responder” because its cognate lesions, thymine dimers and 8-oxoguanines, are likely to be frequently encountered during DNA replication.

5.2. Sequential versus stochastic mechanisms

Here we will discuss how a non-classical polymerase might be chosen when several non-classical polymerases are present in the mutasome. One can imagine a spectrum of possibilities from a purely sequential mechanism on one extreme and a purely stochastic mechanism on the other extreme. In a sequential mechanism, each non-classical polymerase is tried in an established order. For example, pol η , which is typically associated with error-free bypass, may be tried first followed by Rev1 and pol ζ , which are more typically associated with error-prone bypass. By contrast, in a stochastic mechanism, each polymerase is tried in a random order. Here we present the kinetic selection model describing a purely stochastic mechanism and show how such a strategy would maximize efficiency and accuracy.

5.3. The kinetic selection model

This kinetic selection model involves two steps (Fig. 3). In the first step, all of the non-classical polymerases present in the mutasome compete with one another for engaging the damaged DNA substrate. For example, if Rev1 and pol η are both present in the complex, the probability of Rev1 engaging the DNA substrate ($P_{engage(Rev1)}$) is given by:

$$P_{engage(Rev1)} = \frac{k_{engage(Rev1)}}{k_{engage(Rev1)} + k_{engage(pol\ \eta)}}$$

In this equation $k_{engage(Rev1)}$ and $k_{engage(pol\ \eta)}$ are the first-order rate constants for the overall conformational change step leading to Rev1 engaging the DNA substrate and pol η engaging the DNA substrate, respectively. The presence of additional nonclassical polymerases in the mutasome will add more k_{engage} terms to the denominator in this equation.

In the second step, once a non-classical polymerase is engaged with the damaged DNA substrate, there is a competition between incorporating nucleotides opposite the DNA lesion and disengaging from the DNA. For example, if a non-classical polymerase such as Rev1 engages the DNA substrate, the probability of it incorporating a nucleotide opposite the lesion rather than disengaging from the DNA substrate ($P_{incorporate(Rev1)}$) is given by:

$$P_{incorporate(Rev1)} = \frac{k_{incorporate(Rev1)}}{k_{incorporate(Rev1)} + k_{disengage(Rev1)}}$$

In this equation $k_{incorporate(Rev1)}$ is the first-order rate constant for nucleotide incorporation once Rev1 has engaged the DNA substrate and $k_{disengage(Rev1)}$ is the first-order rate constant for Rev1 disengaging from the DNA substrate.

The combined probability that Rev1 will engage the DNA substrate and incorporate nucleotides opposite the damage ($P_{combined(Rev1)}$) is given by:

$$P_{combined(Rev1)} = P_{engage(Rev1)} P_{incorporate(Rev1)}$$

Thus, the probability of Rev1 bypassing the DNA damage ($P_{bypass(Rev1)}$), assuming that Rev1 and pol η are the only two non-classical polymerases in the mutasome, is given by:

$$P_{bypass(Rev1)} = \frac{P_{combined(Rev1)}}{P_{combined(Rev1)} + P_{combined(pol\ \eta)}}$$

The presence of additional non-classical polymerases in the mutasome will add more $P_{combined}$ terms to the denominator in this equation.

5.4. Competition among the polymerases

As mentioned above, the first step of the kinetic selection model involves a competition among all of the non-classical polymerases present in the mutasome for engaging the damaged DNA substrate. For the sake of simplicity, we will consider a competition between Rev1 and pol η . This competition is likely affected by a number of factors including the length and the conformational flexibility in the intrinsically disordered regions of these non-classical polymerases as well as the architecture of the complex (*i.e.*, whether it is a PCNA tool belt or a Rev1 polymerase bridge).

While we do not yet have estimates for the kinetic parameters governing Rev1 and pol η engaging the DNA substrates, we can examine the competition between these non-classical polymerases using BD simulation. We have carried out a series of BD simulations of mutasomes containing PCNA, Rev1, pol η , and a primer-template DNA. Because simulating the mutasome at all atom resolution is not feasible, we carried out coarse-grained (CG) simulations as previously described (22,77,78). In these BD simulations, each amino acid residue was converted to a single CG bead. We carried out 96 independent simulations of the mutasome organized as a PCNA tool belt and 96 independent simulations of the mutasome organized as a Rev1 polymerase bridge (Fig. 4). We allowed the simulations to run up to 500 ns, and we counted the number of simulations in which Rev1 contacted the DNA substrate and the number of simulations in which pol η contacted the DNA substrate.

With respect to PCNA tool belts, Rev1 engaged the DNA substrate in 51 of the 96 simulations (53%), pol η engaged the DNA substrate in 42 of the 96 simulations (44%), and neither polymerase engaged the DNA substrate in 3 of the 96 simulations (3%). With respect to Rev1 polymerase bridges, Rev1 engaged the DNA substrate in 68 of the 96 simulations (71%), pol η engaged the DNA substrate in 21 of the 96 simulations (22%), and neither polymerase engaged the DNA substrate in 7 of the 96 simulations (7%). The simulations strongly suggest that the shorter the tether linking a non-classical polymerase to PCNA, the more likely that polymerase is to engage the DNA substrate. Pol η is far more likely to engage the DNA substrate in a PCNA tool belt, in which it is tethered directly to PCNA, than in a Rev1 polymerase bridge, in which it is tethered to Rev1 which in turn is tethered to PCNA. Thus, the architecture of the mutasome plays a major role in determining which non-classical polymerase has preferential access to the DNA substrate.

5.5. Kinetics of nucleotide incorporation

The second step of the kinetic selection model involves a competition between incorporating a nucleotide opposite the DNA lesion once a non-classical polymerase has engaged the DNA versus disengaging from the DNA substrate. Previous studies of the pre-steady state kinetics of nucleotide incorporation have provided estimates of many of the rate constants necessary to understand this step (79–82). An important caveat is that these kinetic studies were done with individual polymerases on DNA substrates and not in the context of the mutasome. Nevertheless, these parameters represent a reasonable starting point for understanding the second step of the kinetic selection model. These studies suggest that if the DNA lesion is a cognate lesion for the polymerase, the $k_{\text{incorporate}}$ is similar to or greater than the $k_{\text{disengage}}$ and this will favor nucleotide incorporation over disengagement. If the lesion is not a

cognate lesion, $k_{incorporate}$ is significantly less than $k_{disengage}$, and this will greatly favor disengagement over nucleotide incorporation.

For example, in the case of a thymine dimer, a cognate lesion for pol η , the $k_{incorporate}$ is approximately 1 s^{-1} (80). By contrast, for an abasic site, a non-cognate lesion for pol η , the $k_{incorporate}$ is approximately 0.01 s^{-1} (83). The $k_{disengage}$ for the DNA for both lesions is estimated to be about 1 s^{-1} (79). Given these values, if pol η engages a DNA substrate containing a thymine dimer, it would incorporate nucleotides about 50% of the time and disengage from the DNA substrate without incorporating nucleotides about 50% of the time. If it engages a DNA substrate containing an abasic site, it would incorporate nucleotides about 1% of the time and disengage without incorporating nucleotides about 99% of the time. Thus, the competition between incorporating versus disengaging will favor incorporation in the case of cognate lesions and will favor disengaging in the case of non-cognate lesions. In fact, the relative magnitudes of these kinetic parameters are likely the most important factors in determining which non-classical is chosen to bypass the lesion. This means that the relative magnitudes of these kinetic parameters are likely the most important factors for maximizing the accuracy and efficiency of TLS.

6. Conclusions

In this review article, we have discussed how the most appropriate polymerase is chosen. We examined the structural motifs that mediate the protein interactions in the mutasome, the multiple architectures that the mutasome can adopt, the intrinsically disordered regions that tether the polymerases to PCNA and to one another, the assembly of the mutasome, and the kinetic selection model in which the most appropriate polymerase is chosen via a competition among the multiple polymerases within the mutasome. Most of the discussion here was limited to Rev1 and pol η , because these are the best studied of the non-classical polymerases. More work, however, remains to be done. As other components such as pol ζ and perhaps even Rad6-Rad18 are included, additional architectures will need to be considered.

We have also discussed how the kinetic parameters for engaging the DNA substrate, disengaging the DNA substrate, and incorporating nucleotides affects polymerase choice. As mentioned above, estimates for these parameters have come from studies of individual non-classical polymerases. Further kinetic studies in the context of the full mutasome are now needed to understand how interacting partners affect these kinetic parameters. Moreover, determining how post-translational modifications of the non-classical polymerases and other components of the mutasome are also needed to flesh out the kinetic selection model.

The field has made significant progress over the last decade in understanding how the right non-classical polymerase is chosen for TLS. Hopefully additional biochemical, biophysical, and structural studies will be forthcoming in the years ahead to provide us with a more complete understanding of the basis of polymerase choice during TLS.

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Abbreviations

BD	Brownian dynamics
CG	coarse-grained
CTD	C-terminal domain
PCNA	proliferating cell nuclear antigen
PIP	PCNA-interacting protein
Pol ζ	DNA polymerase zeta
Pol η	DNA polymerase eta
SAXS	small-angle X-ray scattering
TIRF	total internal reflection fluorescence
TLS	translesion synthesis
UBM	ubiquitin-binding motif
UBZ	ubiquitin-binding, zinc-binding motif

References

1. Prakash S, Johnson RE and Prakash L (2005) Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. *Annual review of biochemistry*, 74, 317–353.
2. Lehmann AR, Niimi A, Ogi T, Brown S, Sabbioneda S, Wing JF, Kannouche PL and Green CM (2007) Translesion synthesis: Y-family polymerases and the polymerase switch. *DNA repair*, 6, 891–899. [PubMed: 17363342]
3. Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff RV and Walker GC (2009) Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiology and molecular biology reviews : MMBR*, 73, 134–154. [PubMed: 19258535]
4. Washington MT, Carlson KD, Freudenthal BD and Pryor JM (2010) Variations on a theme: eukaryotic Y-family DNA polymerases. *Biochimica et biophysica acta*, 1804, 1113–1123. [PubMed: 19616647]
5. Sale JE, Lehmann AR and Woodgate R (2012) Y-family DNA polymerases and their role in tolerance of cellular DNA damage. *Nature reviews. Molecular cell biology*, 13, 141–152. [PubMed: 22358330]
6. Boiteux S and Jinks-Robertson S (2013) DNA repair mechanisms and the bypass of DNA damage in *Saccharomyces cerevisiae*. *Genetics*, 193, 10251064.
7. Pryor JM, Dieckman LM, Boehm EM and Washington MT (2014) Eukaryotic Y-Family Polymerases: A Biochemical and Structural Perspective. *Nucleic Acids Mol Bi*, 30, 85–108.

8. Hedglin M and Benkovic SJ (2017) Eukaryotic Translesion DNA Synthesis on the Leading and Lagging Strands: Unique Detours around the Same Obstacle. *Chemical reviews*, 117, 7857–7877. [PubMed: 28497687]
9. Friedberg EC, Wagner R and Radman M (2002) Specialized DNA polymerases, cellular survival, and the genesis of mutations. *Science*, 296, 1627–1630. [PubMed: 12040171]
10. Prakash S and Prakash L (2002) Translesion DNA synthesis in eukaryotes: a one- or two-polymerase affair. *Genes & development*, 16, 1872–1883. [PubMed: 12154119]
11. Lawrence CW (2002) Cellular roles of DNA polymerase zeta and Rev1 protein. *DNA repair*, 1, 425–435. [PubMed: 12509231]
12. Powers KT and Washington MT (2017) Analyzing the Catalytic Activities and Interactions of Eukaryotic Translesion Synthesis Polymerases. *Methods Enzymol*, 592, 329–356. [PubMed: 28668126]
13. Johnson RE, Prakash S and Prakash L (1999) Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Pol ϵ . *Science*, 283, 1001–1004. [PubMed: 9974380]
14. Washington MT, Johnson RE, Prakash S and Prakash L (2000) Accuracy of thymine-thymine dimer bypass by *Saccharomyces cerevisiae* DNA polymerase ϵ . *Proceedings of the National Academy of Sciences of the United States of America*, 97, 3094–3099. [PubMed: 10725365]
15. Haracska L, Yu SL, Johnson RE, Prakash L and Prakash S (2000) Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase ϵ . *Nature genetics*, 25, 458–461. [PubMed: 10932195]
16. Washington MT, Minko IG, Johnson RE, Haracska L, Harris TM, Lloyd RS, Prakash S and Prakash L (2004) Efficient and error-free replication past a minor-groove N2-guanine adduct by the sequential action of yeast Rev1 and DNA polymerase zeta. *Molecular and cellular biology*, 24, 69006906.
17. Choi JY and Guengerich FP (2008) Kinetic analysis of translesion synthesis opposite bulky N2- and O6-alkylguanine DNA adducts by human DNA polymerase REV1. *The Journal of biological chemistry*, 283, 23645–23655. [PubMed: 18591245]
18. Wojtaszek J, Liu J, D'Souza S, Wang S, Xue Y, Walker GC and Zhou P (2012) Multifaceted recognition of vertebrate Rev1 by translesion polymerases zeta and kappa. *The Journal of biological chemistry*, 287, 2640026408.
19. Wojtaszek J, Lee CJ, D'Souza S, Minesinger B, Kim H, D'Andrea AD, Walker GC and Zhou P (2012) Structural basis of Rev1-mediated assembly of a quaternary vertebrate translesion polymerase complex consisting of Rev1, heterodimeric polymerase (Pol) zeta, and Pol kappa. *The Journal of biological chemistry*, 287, 33836–33846. [PubMed: 22859295]
20. Boehm EM, Spies M and Washington MT (2016) PCNA tool belts and polymerase bridges form during translesion synthesis. *Nucleic acids research*, 44, 8250–8260. [PubMed: 27325737]
21. Ohmori H, Hanafusa T, Ohashi E and Vaziri C (2009) Separate roles of structured and unstructured regions of Y-family DNA polymerases. *Advances in protein chemistry and structural biology*, 78, 99–146. [PubMed: 20663485]
22. Powers KTE, A. H; Washington MT. (2018) The C-terminal region of translesion synthesis DNA polymerase ϵ is partially unstructured and has high conformational flexibility. *Nucleic acids research*, In Press.
23. Moldovan GL, Pfander B and Jentsch S (2007) PCNA, the maestro of the replication fork. *Cell*, 129, 665–679. [PubMed: 17512402]
24. Zhuang Z and Ai Y (2010) Processivity factor of DNA polymerase and its expanding role in normal and translesion DNA synthesis. *Biochimica et biophysica acta*, 1804, 1081–1093. [PubMed: 19576301]
25. Dieckman LM, Freudenthal BD and Washington MT (2012) PCNA structure and function: insights from structures of PCNA complexes and post-translationally modified PCNA. *Sub-cellular biochemistry*, 62, 281–299. [PubMed: 22918591]
26. Boehm EM, Gildenberg MS and Washington MT (2016) The Many Roles of PCNA in Eukaryotic DNA Replication. *The Enzymes*, 39, 231–254. [PubMed: 27241932]
27. Choe KN and Moldovan GL (2017) Forging Ahead through Darkness: PCNA, Still the Principal Conductor at the Replication Fork. *Molecular cell*, 65, 380–392. [PubMed: 28157503]

28. Jonsson ZO, Hindges R and Hubscher U (1998) Regulation of DNA replication and repair proteins through interaction with the front side of proliferating cell nuclear antigen. *The EMBO journal*, 17, 2412–2425. [PubMed: 9545252]
29. Tsurimoto T (1999) PCNA binding proteins. *Frontiers in bioscience : a journal and virtual library*, 4, D849–858. [PubMed: 10577396]
30. Warbrick E (2000) The puzzle of PCNA's many partners. *BioEssays : news and reviews in molecular, cellular and developmental biology*, 22, 997–1006.
31. Hingorani MM and O'Donnell M (2000) Sliding clamps: a (tail)ored fit. *Current biology : CB*, 10, R25–29. [PubMed: 10660290]
32. Maga G and Hubscher U (2003) Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *Journal of cell science*, 116, 3051–3060. [PubMed: 12829735]
33. Boehm EM and Washington MT (2016) R.I.P. to the PIP: PCNA-binding motif no longer considered specific: PIP motifs and other related sequences are not distinct entities and can bind multiple proteins involved in genome maintenance. *BioEssays : news and reviews in molecular, cellular and developmental biology*, 38, 1117–1122.
34. Hoege C, Pfander B, Moldovan GL, Pyrowolakis G and Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*, 419, 135–141. [PubMed: 12226657]
35. Stelter P and Ulrich HD (2003) Control of spontaneous and damageinduced mutagenesis by SUMO and ubiquitin conjugation. *Nature*, 425, 188191.
36. Bergink S and Jentsch S (2009) Principles of ubiquitin and SUMO modifications in DNA repair. *Nature*, 458, 461–467. [PubMed: 19325626]
37. Shaheen M, Shanmugam I and Hromas R (2010) The Role of PCNA Posttranslational Modifications in Translesion Synthesis. *J Nucleic Acids*, 2010.
38. Ulrich HD and Walden H (2010) Ubiquitin signalling in DNA replication and repair. *Nature reviews. Molecular cell biology*, 11, 479–489. [PubMed: 20551964]
39. Worthylake DK, Prakash S, Prakash L and Hill CP (1998) Crystal structure of the *Saccharomyces cerevisiae* ubiquitin-conjugating enzyme Rad6 at 2.6 Å resolution. *The Journal of biological chemistry*, 273, 6271–6276. [PubMed: 9497353]
40. Notenboom V, Hibbert RG, van Rossum-Fikkert SE, Olsen JV, Mann M and Sixma TK (2007) Functional characterization of Rad18 domains for Rad6, ubiquitin, DNA binding and PCNA modification. *Nucleic acids research*, 35, 5819–5830. [PubMed: 17720710]
41. Parker JL and Ulrich HD (2012) A SUMO-interacting motif activates budding yeast ubiquitin ligase Rad18 towards SUMO-modified PCNA. *Nucleic acids research*, 40, 11380–11388. [PubMed: 23034805]
42. Wang Z, Huang M, Ma X, Li H, Tang T and Guo C (2016) REV1 promotes PCNA monoubiquitylation through interacting with ubiquitylated RAD18. *Journal of cell science*, 129, 1223–1233. [PubMed: 26795561]
43. Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H and Yamaizumi M (2004) Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *The EMBO journal*, 23, 3886–3896. [PubMed: 15359278]
44. Nelson JR, Gibbs PE, Nowicka AM, Hinkle DC and Lawrence CW (2000) Evidence for a second function for *Saccharomyces cerevisiae* Rev1p. *Molecular microbiology*, 37, 549–554. [PubMed: 10931348]
45. Nelson JR, Lawrence CW and Hinkle DC (1996) Deoxycytidyl transferase activity of yeast REV1 protein. *Nature*, 382, 729–731. [PubMed: 8751446]
46. Nair DT, Johnson RE, Prakash L, Prakash S and Aggarwal AK (2005) Rev1 employs a novel mechanism of DNA synthesis using a protein template. *Science*, 309, 2219–2222. [PubMed: 16195463]
47. Nair DT, Johnson RE, Prakash L, Prakash S and Aggarwal AK (2008) Protein-template-directed synthesis across an acrolein-derived DNA adduct by yeast Rev1 DNA polymerase. *Structure*, 16, 239–245. [PubMed: 18275815]

48. Nair DT, Johnson RE, Prakash L, Prakash S and Aggarwal AK (2011) DNA synthesis across an abasic lesion by yeast REV1 DNA polymerase. *Journal of molecular biology*, 406, 18–28. [PubMed: 21167175]
49. Haracska L, Unk I, Johnson RE, Johansson E, Burgers PM, Prakash S and Prakash L (2001) Roles of yeast DNA polymerases delta and zeta and of Rev1 in the bypass of abasic sites. *Genes & development*, 15, 945–954. [PubMed: 11316789]
50. Guo C, Sonoda E, Tang TS, Parker JL, Bielen AB, Takeda S, Ulrich HD and Friedberg EC (2006) REV1 protein interacts with PCNA: significance of the REV1 BRCT domain in vitro and in vivo. *Molecular cell*, 23, 265–271. [PubMed: 16857592]
51. Pustovalova Y, Maciejewski MW and Korzhnev DM (2013) NMR mapping of PCNA interaction with translesion synthesis DNA polymerase Rev1 mediated by Rev1-BRCT domain. *Journal of molecular biology*, 425, 30913105.
52. Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, Kannouche P, Wider G, Peter M, Lehmann AR et al. (2005) Ubiquitinbinding domains in Y-family polymerases regulate translesion synthesis. *Science*, 310, 1821–1824. [PubMed: 16357261]
53. Ohashi E, Murakumo Y, Kanjo N, Akagi J, Masutani C, Hanaoka F and Ohmori H (2004) Interaction of hREV1 with three human Y-family DNA polymerases. *Genes to cells : devoted to molecular & cellular mechanisms*, 9, 523–531. [PubMed: 15189446]
54. Ohashi E, Hanafusa T, Kamei K, Song I, Tomida J, Hashimoto H, Vaziri C and Ohmori H (2009) Identification of a novel REV1-interacting motif necessary for DNA polymerase kappa function. *Genes to cells : devoted to molecular & cellular mechanisms*, 14, 101–111. [PubMed: 19170759]
55. Hishiki A, Hashimoto H, Hanafusa T, Kamei K, Ohashi E, Shimizu T, Ohmori H and Sato M (2009) Structural basis for novel interactions between human translesion synthesis polymerases and proliferating cell nuclear antigen. *The Journal of biological chemistry*, 284, 10552–10560. [PubMed: 19208623]
56. Boehm EM, Powers KT, Kondratieck CM, Spies M, Houtman JC and Washington MT (2016) The Proliferating Cell Nuclear Antigen (PCNA)interacting Protein (PIP) Motif of DNA Polymerase eta Mediates Its Interaction with the C-terminal Domain of Rev1. *The Journal of biological chemistry*, 291, 8735–8744. [PubMed: 26903512]
57. Baranovskiy AG, Lada AG, Siebler HM, Zhang Y, Pavlov YI and Tahirov TH (2012) DNA polymerase delta and zeta switch by sharing accessory subunits of DNA polymerase delta. *The Journal of biological chemistry*, 287, 17281–17287. [PubMed: 22465957]
58. Makarova AV, Stodola JL and Burgers PM (2012) A four-subunit DNA polymerase zeta complex containing Pol delta accessory subunits is essential for PCNA-mediated mutagenesis. *Nucleic acids research*, 40, 11618–11626. [PubMed: 23066099]
59. Makarova AV and Burgers PM (2015) Eukaryotic DNA polymerase zeta. *DNA repair*, 29, 47–55. [PubMed: 25737057]
60. Gomez-Llorente Y, Malik R, Jain R, Choudhury JR, Johnson RE, Prakash L, Prakash S, Ubarretxena-Belandia I and Aggarwal AK (2013) The architecture of yeast DNA polymerase zeta. *Cell Rep*, 5, 79–86. [PubMed: 24120860]
61. Nelson JR, Lawrence CW and Hinkle DC (1996) Thymine-thymine dimer bypass by yeast DNA polymerase zeta. *Science*, 272, 1646–1649. [PubMed: 8658138]
62. Johnson RE, Washington MT, Haracska L, Prakash S and Prakash L (2000) Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. *Nature*, 406, 1015–1019. [PubMed: 10984059]
63. Trincão J, Johnson RE, Escalante CR, Prakash S, Prakash L and Aggarwal AK (2001) Structure of the catalytic core of *S. cerevisiae* DNA polymerase eta: implications for translesion DNA synthesis. *Molecular cell*, 8, 417–426. [PubMed: 11545743]
64. Silverstein TD, Jain R, Johnson RE, Prakash L, Prakash S and Aggarwal AK (2010) Structural basis for error-free replication of oxidatively damaged DNA by yeast DNA polymerase eta. *Structure*, 18, 1463–1470. [PubMed: 21070945]
65. Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S and Aggarwal AK (2010) Structural basis for the suppression of skin cancers by DNA polymerase eta. *Nature*, 465, 1039–1043. [PubMed: 20577207]

66. Biertumpfel C, Zhao Y, Kondo Y, Ramon-Maiques S, Gregory M, Lee JY, Masutani C, Lehmann AR, Hanaoka F and Yang W (2010) Structure and mechanism of human DNA polymerase ϵ . *Nature*, 465, 1044–1048. [PubMed: 20577208]
67. Lawrence CW (2007) Following the RAD6 pathway. *DNA repair*, 6, 676–686. [PubMed: 17254849]
68. McDonald JP, Levine AS and Woodgate R (1997) The *Saccharomyces cerevisiae* RAD30 gene, a homologue of *Escherichia coli* dinB and umuC, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism. *Genetics*, 147, 1557–1568. [PubMed: 9409821]
69. Kannouche P, Broughton BC, Volker M, Hanaoka F, Mullenders LH and Lehmann AR (2001) Domain structure, localization, and function of DNA polymerase ϵ , defective in xeroderma pigmentosum variant cells. *Genes & development*, 15, 158–172. [PubMed: 11157773]
70. Tissier A, Kannouche P, Reck MP, Lehmann AR, Fuchs RP and Cordonnier A (2004) Co-localization in replication foci and interaction of human Y-family members, DNA polymerase pol ϵ and REV1 protein. *DNA repair*, 3, 1503–1514. [PubMed: 15380106]
71. Andersen PL, Xu F, Ziola B, McGregor WG and Xiao W (2011) Sequential assembly of translesion DNA polymerases at UV-induced DNA damage sites. *Molecular biology of the cell*, 22, 2373–2383. [PubMed: 21551069]
72. Garg P and Burgers PM (2005) Ubiquitinated proliferating cell nuclear antigen activates translesion DNA polymerases ϵ and REV1. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 18361–18366.
73. Durando M, Tateishi S and Vaziri C (2013) A non-catalytic role of DNA polymerase ϵ in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks. *Nucleic acids research*, 41, 3079–3093. [PubMed: 23345618]
74. Masuda Y, Kanao R, Kaji K, Ohmori H, Hanaoka F and Masutani C (2015) Different types of interaction between PCNA and PIP boxes contribute to distinct cellular functions of Y-family DNA polymerases. *Nucleic acids research*, 43, 7898–7910. [PubMed: 26170230]
75. Despras E, Delrieu N, Garandeau C, Ahmed-Seghir S and Kannouche PL (2012) Regulation of the specialized DNA polymerase ϵ : revisiting the biological relevance of its PCNA- and ubiquitin-binding motifs. *Environ Mol Mutagen*, 53, 752–765. [PubMed: 23076824]
76. Davies AA, Huttner D, Daigaku Y, Chen S and Ulrich HD (2008) Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein α . *Molecular cell*, 29, 625–636. [PubMed: 18342608]
77. Frembgen-Kesner T and Elcock AH (2009) Striking Effects of Hydrodynamic Interactions on the Simulated Diffusion and Folding of Proteins. *Journal of chemical theory and computation*, 5, 242–256. [PubMed: 26610102]
78. Frembgen-Kesner T and Elcock AH (2010) Absolute protein-protein association rate constants from flexible, coarse-grained Brownian dynamics simulations: the role of intermolecular hydrodynamic interactions in barnase-barstar association. *Biophys J*, 99, L75–77. [PubMed: 21044566]
79. Washington MT, Prakash L and Prakash S (2001) Yeast DNA polymerase ϵ utilizes an induced-fit mechanism of nucleotide incorporation. *Cell*, 107, 917–927. [PubMed: 11779467]
80. Washington MT, Prakash L and Prakash S (2003) Mechanism of nucleotide incorporation opposite a thymine-thymine dimer by yeast DNA polymerase ϵ . *Proceedings of the National Academy of Sciences of the United States of America*, 100, 12093–12098. [PubMed: 14527996]
81. Carlson KD and Washington MT (2005) Mechanism of efficient and accurate nucleotide incorporation opposite 7,8-dihydro-8-oxoguanine by *Saccharomyces cerevisiae* DNA polymerase ϵ . *Molecular and cellular biology*, 25, 2169–2176. [PubMed: 15743815]
82. Pryor JM and Washington MT (2011) Pre-steady state kinetic studies show that an abasic site is a cognate lesion for the yeast Rev1 protein. *DNA repair*, 10, 1138–1144. [PubMed: 21975119]
83. Haracska L, Washington MT, Prakash S and Prakash L (2001) Inefficient bypass of an abasic site by DNA polymerase ϵ . *The Journal of biological chemistry*, 276, 6861–6866. [PubMed: 11106652]

Figure 1. Assembly of the mutasome.

The model of PCNA (*blue*) was obtained from the X-ray crystal structure (1PLQ). The model of full-length Rev1 (*green*) was obtained from X-ray crystal structures of the BRCT domain (4ID3), the polymerase domain (3OSP), and the CTD (3VU7 and 2LSK). The model of pol ζ (*red*) was obtained from the X-ray crystal structure of pol δ (3IAY). The model of pol η (*orange*) was obtained from the X-ray crystal structure the polymerase domain (5VTP). Pol ζ and pol η bind PCNA via their PIP motifs (*insets A and B*). Rev1 binds PCNA via the Rev1 BRCT domain (*inset C*). Pol ζ binds the Rev1 CTD via the Rev7 subunit (*inset D*). Pol η binds the Rev1 CTD via its PIP motif (*inset E*).

Figure 2. Tethering of pol η and Rev1 to PCNA.

An overlay of ten structures of full-length pol η (*orange*) from the ensemble obtained from BD simulations (*left*). These the PIP motifs of these structures have been aligned and placed in the PIP motif-binding site of PCNA (*blue*). An overlay of ten structures of full-length Rev1 (*green*) from the ensemble obtained from BD simulations (*right*). The BRCT domains of these structures have been aligned and placed in the BRCT domain-binding site of PCNA (*blue*).

Figure 3. The kinetic selection model.

A complex containing PCNA (*blue*), Rev1 (*green*), pol η (*orange*), and DNA (*white*) organized as a PCNA tool belt is shown. Initially, neither non-classical polymerase in this complex is engaged with the DNA substrate. Either pol η engages the DNA substrate (*upper pathway*) or Rev1 engages the DNA substrate (*lower pathway*). Once the polymerases have engaged the DNA substrate, they may incorporate one or more nucleotides. The kinetic constants are defined in the text.

Figure 4. BD simulations of PCNA tool belts and Rev1 polymerase bridges.

We carried out 96 BD simulations of PCNA tool belts (*left*) and 96 BD simulations of Rev1 polymerase bridges (*right*). These complexes contained PCNA (*blue*), Rev1 (*green*), pol η (*orange*), and DNA (*white*). In these simulations, each amino acid residue or nucleotide was represented by one CG bead. The simulations were run with a time step of 125 fs, and a pdb file was generated every 1 ns. Each simulation was run for a total of 500 ns. The engagement of a polymerase was defined to be a contact between the primer-terminus of the DNA and any surface of the polymerase that persisted until the end of the simulation. The percentages of simulations in which Rev1 engaged the DNA substrate and in which pol η engaged the DNA substrate are indicated.

Table 1.

Components of the yeast mutasome

Component	Protein/Subunit (Yeast)	Protein/Subunit (Human)	Function
PCNA	Pol30	PCNA	Replication accessory factor; DNA sliding clamp; structurally organizes the mutasome
Rad6-Rad18	Rad6	RAD6A, RAD6B	Ubiquitin-conjugating enzyme (E2); ubiquitylates PCNA
	Rad18	RAD18	Ubiquitin ligase (E3); ubiquitylates PCNA
Rev1	Rev1	REV1	Non-classical DNA polymerase; cognate lesions include minorgroove and exocyclic guanine adducts; structurally organizes the mutasome
Pol ζ	Rev3	REV3	Non-classical DNA polymerase (catalytic subunit); extends from a wide range of DNA lesion
	Rev7	REV7	Non-classical DNA polymerase (accessory subunit)
	Pol31	POLD2	Non-classical DNA polymerase (accessory subunit)
	Pol32	POLD3	Non-classical DNA polymerase (accessory subunit)
Pol η	Rad30	POLH	Non-classical DNA polymerase; cognate lesions include thymine dimers and 8-oxoguanines