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Trading Places: How Do DNA Polymerases Switch during Translesion DNA Synthesis?

Review

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The replicative bypass of base damage in DNA (translesion DNA synthesis [TLS]) is a ubiquitous mechanism for relieving arrested DNA replication. The process requires multiple polymerase switching events during which the high-fidelity DNA polymerase in the replication machinery arrested at the primer terminus is replaced by one or more polymerases that are specialized for TLS. When replicative bypass is fully completed, the primer terminus is once again occupied by high-fidelity polymerases in the replicative machinery. This review addresses recent advances in our understanding of DNA polymerase switching during TLS in bacteria such as *E. coli* and in lower and higher eukaryotes.

Introduction

The rescue of arrested DNA replication at sites of template base damage is critical for cell survival. Several mechanisms for such rescue have been identified in prokaryotic and eukaryotic cells (Lehmann, 2002; Xiao et al., 2000; Hochegger et al., 2004; Friedberg et al., 2005). One general class of damage-tolerance mechanisms is variously referred to as postreplicational repair, postreplicative gap filling, or postreplication recombinational repair. This mechanistic class is characterized by DNA template switching and/or recombination strategies that obviate the need to replicate directly across sites of base damage, hence avoiding the generation of mutations.

An alternative DNA damage tolerance mechanism, called translesion DNA synthesis (TLS), is effected by a recently discovered class of specialized DNA polymerases that support replication directly past template lesions that cannot be negotiated by high-fidelity polymerases. These specialized polymerases can be accurate (error free) or mutagenic (error prone) during TLS. (Friedberg et al., 2002; Goodman, 2002; Hübscher et al., 2002; Lehmann, 2002; Pagès and Fuchs, 2002; Jansen and de Wind, 2003; Rattray and Strathern, 2003). It remains to be established what factors determine whether cells utilize TLS or the first-mentioned class of nonmutagenic tolerance mechanisms in any given situation.

Bacteria such as *E. coli* possess three specialized DNA polymerases (Goodman, 2002; Fuchs et al., 2004), whereas to date ten such enzymes have been identified in mammalian cells (Bebenek and Kunkel, 2004) (Table 1). Though endowed with the prototypic palm, thumb, and fingers domains that characterize all DNA polymerases, structural studies have revealed important differences from the high-fidelity enzymes that support DNA replication (Yang, 2003). In particular, the configuration of the active sites in members of the Y family of specialized polymerases examined to date is more open, a feature that provides a satisfying explanation for their ability to negotiate many types of distortive base damage (Yang, 2003). Consistent with the structural features that endow them with relaxed fidelity when copying damaged DNA, some of the specialized eukaryotic polymerases manifest extremely low fidelity (as low as one error for every 20 nucleotides incorporated) when replicating undamaged DNA (Kunkel et al., 2003). Additionally, all prokaryotic and eukaryotic specialized polymerases lack the exonucleolytic proofreading function that augments the intrinsic high fidelity of their replicative counterparts (Kunkel et al., 2003). Their access to undamaged DNA is thus presumably highly regulated to avoid spurious mutations. Nonetheless, several specialized DNA polymerases have been implicated in somatic hypermutation and/or class switching in the immune system, during which they are believed to generate mutations while copying short stretches of undamaged DNA in immunoglobulin genes (reviewed in Kunkel et al., [2003]).

Notwithstanding their generally relaxed fidelity when copying undamaged DNA, some (if not all) of the eukaryotic specialized polymerases are able to replicate past one or more template lesions with surprising accuracy. For example, human DNA polymerase eta (Polη) preferentially incorporates the correct nucleotide dAMP opposite thymine-thymine cyclobutane pyrimidine dimers (CPD) generated in DNA by exposure to ultraviolet (UV) radiation (Masutani et al., 2000). There are indications that Polη can also accurately bypass other types of base damage (Masutani et al., 2000). The molecular basis for the accuracy of TLS by Polη is not understood but appears to be an intrinsic property of the enzyme (McCulloch et al., 2004). In the absence of Polη (a situation encountered in humans with the variant form of the hereditary skin cancer-prone disease xeroderma pigmentosum [XP]) (Masutani et al., 2000), TLS past CPD is believed to be catalyzed by a different, specialized polymerase(s) and, hence, frequently incorporates incorrect nucleotides, generating the mutations that promote skin cancer (Kannouche and Stary, 2003).

Collectively, these observations have fostered the notion that in higher eukaryotes the multiplicity of spe-

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Table 1. Specialized DNA Polymerases

Bacterial	Yeast (*S. cerevisiae*)	Humans	Polymerase Family
Pol II[a]			B
Pol IV			Y
Pol V			Y
	Rev 1	Rev 1	Y
	Pol ζ	Pol ζ	B
	Pol η	Pol η	Y
		Pol κ	Y
		Pol ι	Y
		Pol λ	X
		Pol μ	X
		Pol β	X
		Pol θ	A
		Pol ν	A

[a] Unlike Pol IV and Pol V, Pol II of *E. coli* has intrinsic 5′→3′ exonuclease activity but is included in this table because it is implicated in TLS.

cialized DNA polymerases reflects the evolution of different enzymes to accurately negotiate different types of naturally occurring base damage (Friedberg et al., 2002). The additional observation that these polymerases can support TLS extending past many other types of base damage in vitro, albeit inaccurately, suggests that in the absence of the "correct" polymerase for a particular lesion or class of lesions, another polymerase(s) can substitute, thereby promoting cell survival but with an increased probability of generating mutations (Friedberg et al., 2002).

A proven functional relationship of a particular eukaryotic specialized DNA polymerase to a particular type of base damage is presently restricted to Polη and the bypass of thymine-thymine CPD. However, the availability of mouse strains defective in genes that encode other specialized polymerases (including Polκ [Schenten et al., 2002], Polι [McDonald et al., 2003], Polμ [Bertocci et al., 2002] and Polλ [Bertocci et al., 2002]) offers the promise of revealing further functional relationships.

Implicit in any model of TLS is the notion that at different stages of the process different DNA polymerases occupy the primer terminus at or near to sites of arrested replication (polymerase switching) (Plosky and Woodgate, 2004) (Figure 1). In the first instance, the high-fidelity DNA polymerases in the replication machinery arrested at the primer terminus must be replaced with a specialized polymerase that can catalyze the incorporation of a limited number of nucleotides directly opposite the arresting lesion (Figure 1). In vitro studies indicate that some eukaryotic specialized polymerases are well suited to incorporating nucleotides directly opposite lesions (TLS insertion) but weakly extend synthesis beyond the lesion (TLS extension) (Prakash and Prakash, 2002). Other specialized polymerases appear to be better suited to extending distorted primer termini after insertion has taken place. Such extension is presumably critical, because regardless of whether nucleotide incorporation opposite sites of base damage is accurate or not, a perfectly base-paired structure will not be generated, and this will preclude productive reengagement of the replicative machinery, as discussed below. Bacteria such as *E. coli* apparently employ a single specialized polymerase for both TLS insertion and TLS extension. However, in at least some instances of TLS in eukaryotes, a second polymerase switch between "insertion" and "extension" enzymes may be required (Figure 1), though the generality of this so-called two-polymerase model remains to be definitively demonstrated. When the primer has been extended to a position at which newly incorporated nucleotides are no longer susceptible to removal by exonucleolytic proofreading, another polymerase switch must take place in both prokaryotes and eukaryotes to reengage the replicative machinery in high-fidelity replication (Figures 1 and 2).

The mechanism(s) by which these polymerase switches transpire is poorly understood and poses a critical challenge to comprehending the process of TLS and its contribution to spontaneous and environmentally induced mutagenesis in cells. Such comprehension must surely include answers to at least the following questions. (1) How is a particular specialized DNA polymerase(s) that supports accurate TLS across a particular type of base damage selected? (2) How is the exchange between the arrested replication machinery and this specialized insertion polymerase effected? (3) How is the exchange between insertion and extension polymerases orchestrated in eukaryotes? (4) How does the replicative machinery reengage the primer template once TLS is completed? (5) How is the access of specialized polymerases to native DNA restricted? Recent studies discussed in this review have begun to provide some potentially important insights into these questions.

DNA Polymerase Switching during TLS in *Escherichia coli*

It is well established that mutations are generated at or near sites of template base damage (targeted mutagenesis) during replication of damaged DNA. In *E. coli*, such targeted mutagenesis requires the products of the *umuDC*, *recA,* and *dnaN* genes (Kato and Shinoura, 1977; Steinborn, 1978; Blanco et al., 1982; Dutreix et al., 1989; Sweasy et al., 1990; Becherel et al., 2002). The *umuDC* locus encodes the specialized DNA polymerase (Pol V) directly involved in TLS (Reuven et al., 1999; Tang et al., 1999). RecA protein and the β clamp replication processivity factor (the *dnaN* gene product) act as accessory factors that are essential for the Pol V-mediated bypass reaction (see below). Pol V can bypass multiple template lesions, including CPD, T(6-4)T photoproducts, AP sites, and G-AAF adducts, with similar efficiency (Reuven et al., 1999; Tang et al., 2000; Fujii and Fuchs, 2004). In contrast to the situation encountered in eukaryotes, which use different specialized polymerases at the insertion and extension steps, Pol V appears to perform both reactions (see below).

Efficient Pol V-mediated bypass requires RecA protein and the β clamp stably loaded onto the primer template (Figure 2) (Goodman, 2002; Fujii et al., 2004). In some instances, TLS in prokaryotes such as *E. coli* utilizes the specialized polymerase Pol IV (the product of the *dinB* gene) or Pol II (the product of the *polB* gene) (Napolitano et al., 2000; Wagner et al., 2002; Shen et al., 2002). Recently, polymerase switching between Pol V and the Pol III holoenzyme arrested at a defined site

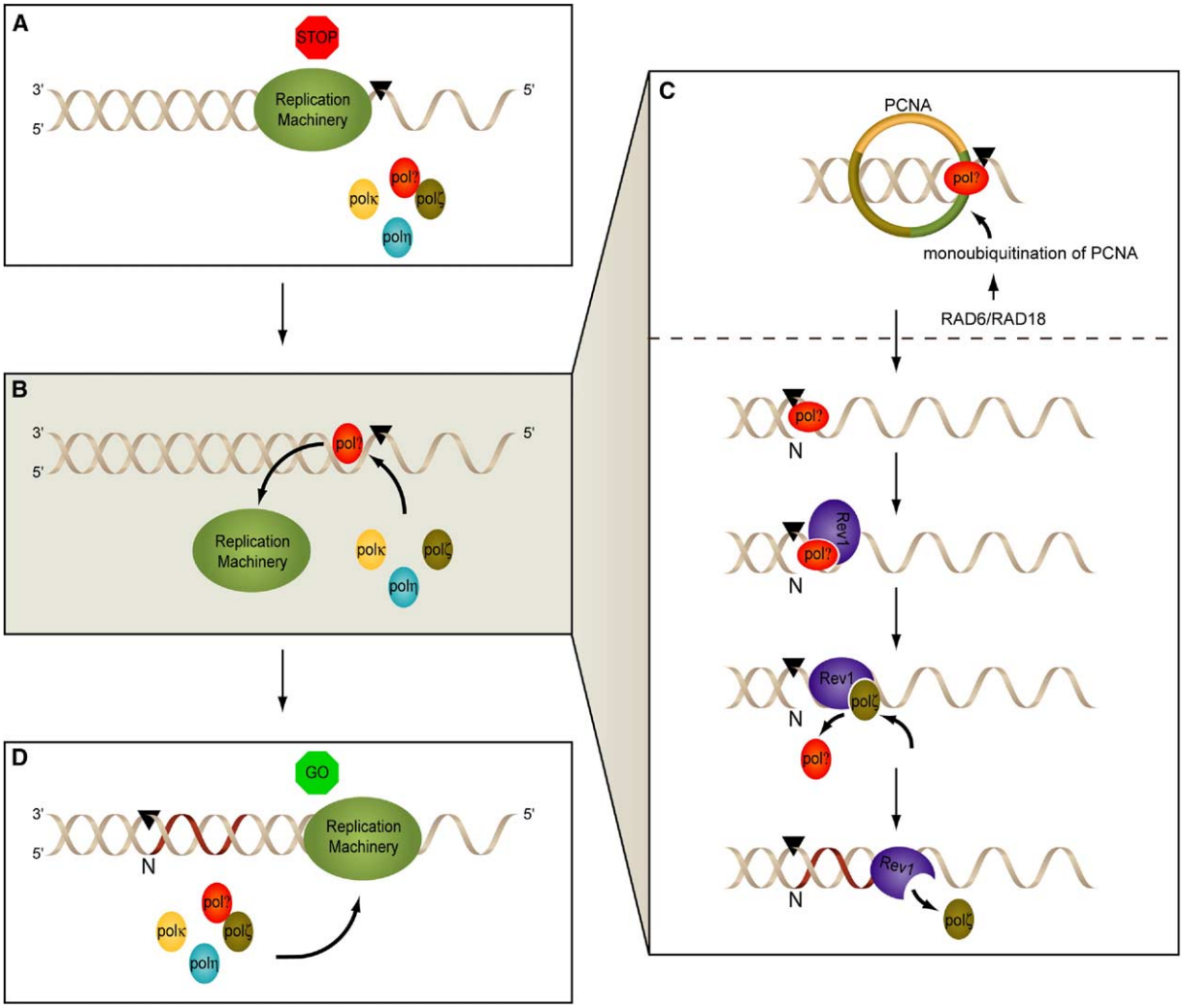

Figure 1. A Model for Translesion DNA Synthesis in Mammalian Cells Exposed to UV Radiation

(A) High-fidelity DNA replication (replication machinery) is shown arrested at a generic form of base damage (inverted triangle). Some of the multiple specialized DNA polymerases (polη, polκ, polζ, and one designated generically as "pol?") are depicted in the general proximity of the arrested replication fork. However, their definitive intranuclear localization in relation to normal and arrested replication is unknown.

(B) A switch between polδ or polε in the arrested replicative machinery and a selected specialized DNA polymerase (in this case, pol?) is shown.

(C) Top: switching in pol? to bypass the lesion may require monoubiquitination of the trimeric PCNA clamp, supported by the RAD6/RAD18 ubiquitin ligase. (C) Bottom: after a correct residue (N) is incorporated by pol?, Rev1 protein may be involved in a switch between the insertion polymerase (pol?) and an extension polymerase, such as polζ. The patch of DNA synthesis generated in this two-polymerase model is shown in red. For clarity, the continued involvement of PCNA is not shown in the lower part.

(D) When TLS past the lesion has extended to a suitable position downstream of the lesion, a third polymerase switch transpires during which the replicative machinery is again productively engaged with the primer terminus and high-fidelity DNA replication continues.

of base damage in circular DNA molecules was reconstituted in vitro (Fujii and Fuchs, 2004). This in vitro system recapitulates the genetic requirements for DNA damage-induced mutagenesis in *E. coli* (Sutton et al., 2000; Goodman, 2002; Fuchs et al., 2004). The long regions of single-stranded DNA generated downstream of a template lesion as a consequence of the transient uncoupling of the coordinated leading- and lagging-strand synthesis (Pagès and Fuchs, 2003) are expected to generate extended RecA filaments in vivo. In the study by Fujii and Fuchs (2004), TLS is accompanied by the assembly of an extended RecA-ATP filament on the single-stranded template DNA generated downstream of the lesion as a consequence of transient uncoupling of the coordinated leading- and lagging-strand synthesis (Pagès and Fuchs, 2003) as well as the stable loading of the ring-shaped β clamp on the primer template (Figure 2). However, other studies that used shorter oligonucleotides showed that a RecA filament is not required for TLS (Schlacher et al., 2005). The study by Schlacher et al. (2005) also suggests that two molecules of RecA bound to Pol V in the presence of DNA are minimally necessary to catalyze TLS.

In the study by Fujii and Fuchs (2004), Pol III holoenzyme arrests at the nucleotide immediately preceding the damaged base (position L-1) at all blocking lesions

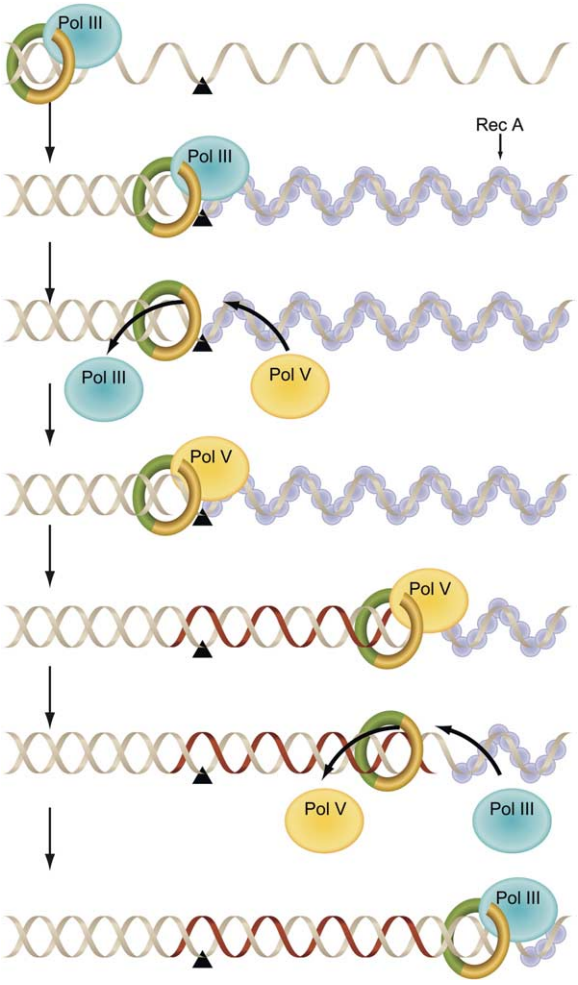

Figure 2. A Model for TLS in a Prokaryote Such as *E. coli*

The replicative polymerase (Pol III) associated with the β clamp processivity factor (green and yellow circle) reaches a noncoding lesion (shown as a triangle) and arrests at the nucleotide immediately preceding a damaged base. A region of single-stranded DNA that forms downstream from the lesion site as a consequence of the transient progression of the replicative helicase (Pagès and Fuchs, 2003) triggers the formation of an extended RecA filament. Pol V binds to the 3′-OH terminus of the primer and forms a stable complex by means of its dual interaction with both the RecA filament and the β clamp. (Other studies [Schlacher et al., 2005] do not indicate an absolute requirement for the RecA filament.) In the model shown here, the "Pol V-RecA-β-clamp" complex loaded onto the primer terminus allows Pol V to synthesize a patch of DNA about 20 nucleotides long on average (shown in red). Pol V produces a large distribution of TLS patches ranging from 1–60 nucleotides (Fujii and Fuchs, 2004). If the TLS patch is ≥5 nucleotides, the distortion created by the lesion lies outside the "sensor domain" of Pol III, allowing efficient high-fidelity extension of the primer, and, thus, successfully completing TLS. In contrast, if the TLS patch is <5 nucleotides, the distortion triggers primer degradation by the proofreading function associated with Pol III, leading to aborted TLS.

thus far examined. The switch between the replicative and TLS polymerases is effected by the β clamp, which dissociates from the arrested replicative machinery. The β clamp then becomes available for interaction with Pol V, which catalyzes a short patch of DNA synthesis (TLS patch) extending from directly opposite to several nucleotides downstream from the lesion (Fujii and Fuchs, 2004).

Purified Pol V is highly distributive when copying naked damaged DNA in vitro. However, when the enzyme is bound to the β clamp encircling the primer template and the single-stranded template DNA is appropriately coated with RecA protein, Pol V is more processive. Hence, in contrast to the situation in eukaryotes, in which a second specialized polymerase is thought to be required for primer extension beyond the site of base damage (see below), *E. coli* Pol V can efficiently catalyze both nucleotide insertion and extension in vitro.

The size distribution of the TLS patches ranges from 1–60 nucleotides, with an average size of 18 nucleotides (Figure 2) (Fujii and Fuchs, 2004). About 75% of the Pol V-mediated TLS patches are longer than five nucleotides (Fujii and Fuchs, 2004). Although Pol V is error prone when incorporating nucleotides directly opposite sites of base damage, its fidelity when copying undamaged DNA is such that it is not expected to generate additional untargeted mutations despite the relatively large size of the average TLS patch (Fujii and Fuchs, 2004).

If the TLS patch is extended for less than 4 bp past the lesion in this in vitro system, Pol III holoenzyme can degrade the nascent primer to position L-1 through its associated 3′ → 5′ exonuclease activity, essentially negating the bypass reaction (Figure 2). In contrast, primers extended five nucleotides or more beyond the lesion are readily occupied by Pol III holoenzyme and are further extended with high fidelity (Fujii and Fuchs, 2004). It remains to be determined whether additional factors determine the dissociation of Pol V and the reengagement of the Pol III holoenzyme after TLS is completed. Nonetheless, the in vitro events just described provide a reasonable working model of polymerase switching in prokaryotes and explain the ability of a single specialized DNA polymerase (Pol V) to effect both DNA insertion and DNA extension during TLS.

In eukaryotes, the switch between replicative and TLS polymerases appears to be influenced by monoubiquitination of proliferating cell nuclear antigen (PCNA), the structural and functional homolog of the bacterial β processivity clamp (see below). No specific posttranslational modification of the β clamp has been observed in bacteria. However, there are indications that the interaction of UmuC with the β clamp may be modulated by differential interactions with its partners UmuD and UmuD′ (Duzen et al., 2004), and this may serve an analogous function to the posttranslational modification of PCNA.

DNA Polymerase Switching during TLS in Eukaryotes

The process of polymerase switching during TLS in eukaryotes is less well understood. However, recent studies have identified several features that appear to contribute to the multiple polymerase switching events illustrated diagrammatically in Figure 1.

Posttranslational Modification of Proteins Associated with TLS

A major difference between prokaryotes and eukaryotes is the widespread use by the latter of posttranslational protein modifications as a general mechanism for regulating protein activity and for transmitting signals in response to DNA damage. Transmission of cell cycle-checkpoint signals is mediated by numerous phosphorylation events. More recently, several examples of protein ubiquitination and modification by the small ubiquitin-related modifier (SUMO) in response to DNA damage have been discovered, notably of PCNA (Hoege et al., 2002; Stelter and Ulrich, 2003). In the yeast *S. cerevisiae*, genes in the *RAD6* epistasis group regulate the replication of damaged DNA. Several members of this group of genes have been shown to encode E2 ubiquitin-conjugating enzymes or E3 ubiquitin ligases. Rad6 protein is a well-characterized E2 enzyme (Jentsch et al., 1987) that associates with the E3 ligase Rad18 (Bailly et al., 1997). Similarly, Ubc13-Mms2 is a heterodimeric E2 enzyme that forms ubiquitin chains via Lys63 linkages (Hoffman and Pickart, 1999) (rather than the more common Lys48 linkages used for targeting proteins for degradation by the proteasome), and Rad5 is yet another chromatin-associated E3 ligase. Rad18 protein binds to single-stranded DNA, interacts with Rad6 and Rad5 proteins, and can homodimerize, although both self association of Rad18 and its interaction with Rad5 are relatively weak. Rad5 protein interacts with both Rad18 and Ubc13-Mms2 and can also form homodimers (Ulrich and Jentsch, 2000).

Genetic studies indicate that the pathway mediated by the Mms2-Ubc13 E2 enzyme is error free, whereas it has been known for many years that Rad6 and Rad18 are additionally required for an error-prone pathway(s) that leads to UV radiation-induced mutagenesis (Lawrence, 1994). Hence, an emerging model from studies with yeast is that Rad6 and Rad18 are involved in both recombination and/or copy choice and TLS mechanisms for tolerating unrepaired base damage in eukaryotes (Xiao et al., 2000).

The protein target for the ubiquitination reactions mediated by the Rad6-Rad18 and Mms2-Ubc13-Rad5 ubiquitin ligases has been identified as the polymerase sliding-clamp PCNA, the functional homolog of the bacterial β processivity clamp (Hoege et al., 2002) and a protein that interacts with many players on the DNA metabolism stage (Maga and Hübscher, 2003). When replicating cells are exposed to DNA damage, PCNA is monoubiquitinated at Lys164 by the concerted action of Rad6 and Rad18; whereas, polyubiquitination is effected by Mms2-Ubc13 (E2) and Rad5 (E3). These results, together with supporting genetic data (Xiao et al., 2000; Stelter and Ulrich, 2003), suggest that stalling of the replication machinery at sites of DNA damage results in monoubiquitination of PCNA, which directs the replication machinery into the TLS pathway, that can be either error free or error prone. Polyubiquitination by the addition of further lys63-linked ubiquitin molecules channels DNA lesions into the error-free recombinational pathway(s). In human cells, only monoubiquitination has been detected to date, this being effected by the human homologs of yeast Rad6 and Rad18 (Kannouche et al., 2004; Watanabe et al., 2004).

Several laboratories have examined the effect of monoubiquitination of PCNA. Two groups have reported that this posttranslational modification in the chromatin of UV-irradiated cells increases the affinity of chromatin for the specialized polymerase Polη (Kannouche et al., 2004; Watanabe et al., 2004). These observations provide an attractive model for effecting the first polymerase switch, from replicative to TLS insertion polymerase (at least for Polη during the bypass of photoproducts in DNA), during which the increased affinity of ubiquitinated PCNA for Polη results in a "switching out" of the replicative enzymes Polδ or Polε and the "switching in" of Polη (Figure 1). As yet there is no direct evidence as to whether or not monoubiquitination of PCNA specifically lowers its affinity for the replicative polymerases.

Posttranslational monoubiquitination of PCNA occurs not only in response to DNA damage but also after exposure of cells to hydroxyurea (Kannouche et al., 2004), an agent that stalls DNA replication by depleting deoxyribonucleotide pools. It therefore seems likely that the ubiquitination process is triggered by arrested DNA replication per se, rather than the presence of template base damage at the replication fork. In the case of CPDs generated by exposure to UV light, switching in Polη is expected to be productive, enabling accurate TLS insertion of two nucleotides. However, such polymerase switching serves no obvious purpose when cells are exposed to hydroxyurea or to replication-blocking lesions that cannot be bypassed by Polη. A related conundrum derives from the question as to how any given polymerase is selected for TLS extending past any given type of base damage, as exemplified by the use of Polη to bypass CPD. Both Polι and Polκ have PCNA binding motifs, but there is no evidence as yet that their affinity for PCNA is similarly increased by monoubiquitination after exposure to specific types of DNA-damaging agents. Perhaps different polymerases have varying affinities for specific types of base damage, and this property helps select the appropriate enzyme once the replicative machinery has disengaged. Additionally, or alternatively, the amount of a given specialized polymerase may be upregulated by transcriptional and/or posttranscriptional events in response to exposure of cells to certain DNA-damaging agents, facilitating specialized polymerase selection by mass action. Finally, the specialized polymerases may themselves undergo specific types of posttranslational modification that facilitate their selection for TLS.

Once a specialized polymerase has incorporated the required number of nucleotides opposite a lesion, it is likely to dissociate because of the inherently poor processivity of these enzymes and the inefficiency with which some of them extend mismatched primer templates. Indeed it has been demonstrated in vitro that Polη has a decreased probability of dissociating when it incorporates nucleotides opposite a thymine-thymine CPD but an increased probability of dissociating once it has progressed a few nucleotides beyond the lesion (McCulloch et al., 2004). It remains an open question whether or not PCNA must be specifically deubiquitinated in vivo to facilitate such dissociation and/or further polymerase switches. It also remains to be established which factors determine whether PCNA is

monoubiquitinated for a function(s) in TLS or is polyubiquitinated for a function(s) in error-free DNA damage tolerance, and what the specific role of polyubiquitination is in the latter scenario.

Possible Role(s) of Rev1 Protein in Polymerase Switching

Rev1 protein is a member of the Y family of specialized DNA polymerases (Lawrence, 2004). In contrast to the other specialized polymerases, not all of which are represented in all eukaryotes, Rev1 is ubiquitous. Rev1 has a restricted DNA polymerase activity that is confined to the incorporation of one or two molecules of dCMP regardless of the nature of the template nucleotide (Lawrence, 2004). The protein is thus a dCMP transferase. The biological function of the dCMP transferase is presently uncertain, though there are indications that this catalytic activity is employed for TLS past sites of base loss (AP sites) (Lawrence, 2004). Importantly, however, inactivation of the dCMP transferase of yeast Rev1 by site-directed mutagenesis does not abrogate its requirement for UV radiation-induced mutagenesis in yeast (Otsuka et al., 2002; Lawrence, 2004). Assuming that UV radiation-induced mutagenesis in cells primarily, if not exclusively, reflects a function of Rev1 in TLS, these observations suggest a role(s) for Rev1 that is independent of the dCMP activity.

Several recent studies in both human and mouse systems have demonstrated that Rev1 protein interacts with multiple TLS polymerases, notably Polη, Polκ, Polι, Polλ, and the REV7 subunit of the heterodimeric REV7-REV3 complex that comprises Polζ (Guo et al., 2003; Tissier et al., 2004; Ohashi et al., 2004). However, Rev1 protein does not interact with several other low-fidelity polymerases examined, such as Polβ or Polμ, indicating that it is discriminate in its polymerase interactions. All these polymerase interactions require just the C-terminal 100 amino acids of the Rev1 protein. Additionally, competition experiments have shown that binding of Rev1 to a fixed amount of Polκ can be competed by increasing amounts of Rev7 protein in vitro (Guo et al., 2003), i.e., Rev1 can switch between one specialized polymerase and another by mass action. Collectively, these observations raise the interesting possibility that Rev1 protein may be specifically involved in polymerase switching during TLS. A priori this function may involve switching between the replicative machinery and one or more insertion polymerases or between an insertion polymerase and an extension polymerase.

Rev1 protein from *S. cerevisiae* or *C. elegans* does not interact directly with Polη from these organisms in the yeast two-hybrid assay or with mouse or human Polη. Furthermore, in contrast to higher eukaryotes in which the C-terminal 100 amino acids of Rev1 are highly conserved, the conservation of these amino acids in Rev1 from lower eukaryotes is considerably reduced. Whereas the possibility that one or more other proteins are required for these interactions in vivo remains to be explored, one must consider the possibility that the role of Rev1 in TLS differs fundamentally between lower and higher organisms.

In conclusion, it is apparent that the multiple polymerase switching events that occur during TLS involve several contributing mechanisms that regulate the access of low-fidelity, error-prone enzymes to DNA. In prokaryotes, these factors include the β clamp, RecA protein, and RecA-DNA filaments. In eukaryotes, they include ubiquitination of PCNA and possibly of other proteins, including the polymerases themselves. Additionally, TLS extending past many types of altered bases in eukaryotes requires Rev1 protein, perhaps in a role(s) that exploits its ability to interact with multiple specialized polymerases and possibly with other proteins.

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