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A novel variant of DNA polymerase ζ, Rev3ΔC, highlights differential regulation of Pol32 as a subunit of polymerase δ *versus* ζ in *Saccharomyces cerevisiae*

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ABSTRACT

Unrepaired DNA lesions often stall replicative DNA polymerases and are bypassed by translesion synthesis (TLS) to prevent replication fork collapse. Mechanisms of TLS are lesion- and species-specific, with a prominent role of specialized DNA polymerases with relaxed active sites. After nucleotide(s) are incorporated across from the altered base(s), the aberrant primer termini are typically extended by DNA polymerase ζ (pol ζ). As a result, pol ζ is responsible for most DNA damage-induced mutations. The mechanisms of sequential DNA polymerase switches *in vivo* remain unclear. The major replicative DNA polymerase δ (pol δ) shares two accessory subunits, called Pol31/Pol32 in yeast, with pol ζ. Inclusion of Pol31/Pol32 in the pol δ/pol ζ holoenzymes requires a [4Fe–4S] cluster in C-termini of the catalytic subunits. Disruption of this cluster in Pol ζ or deletion of *POL32* attenuates induced mutagenesis. Here we describe a novel mutation affecting the catalytic subunit of pol ζ, *rev3ΔC*, which provides insight into the regulation of pol switches. Strains with Rev3ΔC, lacking the entire C-terminal domain and therefore the platform for Pol31/Pol32 binding, are partially proficient in Pol32-dependent UV-induced mutagenesis. This suggests an additional role of Pol32 in TLS, beyond being a pol ζ subunit, related to pol δ. In search for members of this regulatory pathway, we examined the effects of Maintenance of Genome Stability 1 (Mgs1) protein on mutagenesis in the absence of Rev3–Pol31/Pol32 interaction. Mgs1 may compete with Pol32 for binding to PCNA. Mgs1 overproduction suppresses induced mutagenesis, but had no effect on UV-mutagenesis in the *rev3ΔC* strain, suggesting that Mgs1 exerts its inhibitory effect by acting specifically on Pol32 bound to pol ζ. The evidence for differential regulation of Pol32 in pol δ and pol ζ emphasizes the complexity of polymerase switches.

1. Introduction

Cellular DNA is under constant attack by exogenous and endogenous mutagens. Resulting lesions, if unrepaired [1,2], can block cell division when replicative DNA polymerases are unable to incorporate nucleotides across from the damaged sites. This causes activation of DNA damage tolerance mechanisms to prevent irreversible replication fork collapse and to finish replication of the genome [3]. The DNA damage tolerance pathway includes predominantly error-free recombinational damage avoidance and translesion synthesis (TLS), which often is a source of mutations [4,5]. While TLS machinery deals with the lesion, the replication fork can restart downstream to allow for continuation of replication [6].

During TLS, replicative DNA polymerases yield the damaged template to specialized polymerases which incorporate nucleotides across from the altered base(s) [7–9]. Most prominent in TLS are the low fidelity Y-family polymerases η, κ, ι, and Rev1 [10,11], but in some cases insertion is accomplished by X-family, A-family, or B-family DNA polymerases [5,12]. Then there is extension of the aberrant primer terminus, achieved by the inserter or another polymerase. Most frequently this extension is accomplished by the error-prone B-family polymerase ζ (pol ζ) [4,12–14]. Once the lesion is bypassed, there is a return to

Abbreviations: CTD, C-terminal domain; FeS, [4Fe–4S] iron sulfur; Mgs1, Maintenance of Genome Stability 1; ZnF, zinc finger.

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synthesis by replicative polymerases or filling of the gap between the bypassed lesion and a downstream restart site, by pol ζ itself or by replicative polymerases [5,6].

TLS events can have opposing effects on mutagenesis. Some TLS polymerases are tailored to bypass specific types of lesions and incorporate predominantly the correct base, *i.e.* the base that should have been incorporated by the replicative polymerase in the absence of damage. Historically, this is called error-free bypass because the action of these polymerases suppresses induced mutagenesis. However, the number of lesions greatly exceeds the number of polymerases. Therefore most lesions are primarily bypassed by the addition of an incorrect base. This so-called error-prone TLS is highly mutagenic. This process is carried out by a complex of proteins composed of replicative pols, TLS pol ζ, Rev1, and monoubiquitylated proliferating cell nuclear antigen (PCNA) [4,9,15,16].

One critical event during TLS in eukaryotes is the physical switch between the polymerases. Details of how it actually occurs *in vivo* are not clear. Currently it is thought that it occurs *via* the two-step insertion–extension mechanism, proposed on the basis of experiments in yeast (Fig. 1A) [11,12,17]. Upon damage, PCNA is monoubiquitylated at K164 [18] and there is a switch from replicative pol δ (or pol ε) to another polymerase (predominantly Y-family pol) which inserts a nucleotide across from the lesion. Rev1 acts as an indispensable scaffold protein and, when necessary, a deoxycytidyl transferase inserting "C" opposite the lesion. Then there is a switch to pol ζ which performs extension from this aberrant terminus. If an error was made during bypass, the action of pol ζ allows the altered sequence to remain in the nascent DNA strand sequence, leading to a mutation. Malfunction of this pathway abolishes induced mutagenesis. The signals involved, aside from ubiquitylation and probably deubiquitylation of PCNA, are unknown [19,20].

Pol ζ is responsible for most induced point mutations and roughly half of spontaneous mutations [9,21]. It synthesizes DNA *in vitro* with low fidelity and produces a characteristic mutational signature [22], found in mutation spectra *in vivo* [2,23,24]. Part of the signature is attributed to template switches [25]. Pol ζ is the only TLS polymerase essential for viability in mice, suggesting it is required for tolerance of endogenous DNA damage during development. In yeast, deletion of *REV3* is not lethal but causes growth retardation in strains with elevated levels of abasic sites [26]. Loss of the catalytic subunit of pol ζ or Rev1 results in elevated rates of large deletions [24,25] and gross chromosomal abnormalities [27]. Therefore, while error-prone TLS is etiologic in most environmentally induced cancers, its absence can also contribute to genome instability and cancer [13,28,29]. Pol ζ can also contribute to cancer cell resistance to the chemotherapeutic agent cisplatin [30].

Pol ζ was long thought to be composed of only Rev3 and Rev7 [31]. We discovered that the C-terminal domain (CTD) of the human catalytic subunit of pol ζ binds two accessory subunits of pol δ, p50/p66, and predicted that human pol ζ is a four-subunit complex (See Table 1 for nomenclature of human and yeast DNA polymerase subunits) [32]. Four-subunit human pol ζ was later purified from human cells and possessed polymerase activity superior to the two-subunit enzyme [33]. Yeast pol ζ can also stably exist as a four-subunit enzyme, containing the catalytic subunit Rev3, accessory subunit Rev7, and Pol31/Pol32 [34–36]. In this complex, Pol32 binds to Pol31, and Pol31 binds to the CTD of catalytic subunit Pol3 [37–39]. The existence of shared subunits between replicative and TLS pols was the basis for the proposal of an additional mechanism of switching between pol δ and pol ζ through an exchange of the catalytic subunits on Pol31/Pol32 bound to PCNA [32]. In this scenario (Fig. 1B), pol δ stalling at a lesion signals for monoubiquitylation of PCNA. Then the catalytic subunit Pol3 dissociates (and/or is degraded [40]) and Rev3/Rev7 is recruited to Pol31/Pol32 left at the site of the lesion. This mechanism provides an easy, yet unproven, possibility for a switch back to Pol3 for processive synthesis if necessary (more in Section 4, Discussion). In this model, pol δ plays a role in TLS by regulating the entire switch process.

Table 1
Nomenclature for yeast and human Pol δ and Pol ζ.

Organism	Subunit	Gene	Protein
Polymerase δ			
Yeast	Catalytic	*POL3*	Pol3
	B	*POL31*	Pol31
	C	*POL32*	Pol32
Human	Catalytic	*POLD1*	p125
	B	*POLD2*	p50
	C	*POLD3*	p66
	Small 4th	*POLD4*	p12
Polymerase ζ			
Yeast	Catalytic	*REV3*	Rev3
	Accessory	*REV7*	Rev7
	B, C	*POL31*, *POL32*	Pol31, Pol32
Human	Catalytic	*REV3L*	p353
	Accessory	*REV7*	P30
	B, C	*POLD2*, *POLD3*	P50, p66

It is believed that based on the structure of another B-family member pol α and a low resolution EM structure of pol ζ, that both Pol3 and Rev3 contain a CTD attached by a flexible linker [39,41]. Both polymerases contain a FeS cluster in this domain [42], which is required for binding to Pol31/Pol32 [32,34,35]. In addition, when the C-terminal tail of Rev3 past the metal binding sites is removed there is no binding to Pol31, suggesting that not only is the cluster necessary for binding but the region of the CTD downstream of it is also [34]. In yeast when the FeS cluster of pol ζ is disrupted, there is a severe reduction of mutagenesis comparable to the complete absence of Rev3 ([32,34], present study). It is possible that this cluster plays a structural role or that the switch is regulated by oxidation–reduction reactions [43]. To better understand how polymerase switches occur in yeast, we created several mutants affecting the CTD of Rev3 (Fig. 2A). Intriguingly, even though disruption of the cluster nearly eliminated induced mutagenesis, a mutant that lacked the entire CTD and thus the whole platform for interaction with Pol31/Pol32, exhibited robust mutagenesis at low doses of UV irradiation and residual mutagenesis at higher doses. The purpose of this study was to characterize this C-terminal truncation mutant, *rev3ΔC*, and use it as a tool to probe our understanding of polymerase switches *in vivo*.

We found that UV-induced mutagenesis in this mutant still requires the presence of Pol32 but becomes independent of regulation by Maintenance of Genome Stability 1 (Mgs1), whose overproduction suppresses mutagenesis in strains with normal pol ζ [44]. We conclude that Pol32 plays a role in mutagenesis beyond its function as subunit of pol ζ and that Mgs1 is capable of regulating UV-induced mutagenesis only when the Pol31/Pol32 binding platform in Rev3 is intact.

2. Materials and methods

2.1. Materials

Most mutagenesis studies were done in the *Saccharomyces cerevisiae* strain 8C-YUNI101 (*MAT***a** *his7-2 leu2-3,112 ura3-Δ bik1*::*ura3-29RL trp1-1*$_{UAG}$ *ade2-1*$_{UAA}$) [45] and its derivatives. Mutagenesis studies on the deletion of *MGS1* were done in a derivative of the strain BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0; ura3Δ0*) (Life Technologies, USA). Extracts for western blotting were prepared from transformants of the protease-deficient strain BJ2168 (*MATa prc1-407 prb1-1122 pep4-3 leu2 trp1 ura3-52 gal2*) [46]. Plasmids used are described

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