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Lesion bypass by *S. cerevisiae* Pol ζ alone

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ABSTRACT

DNA polymerase zeta (Pol ζ) participates in translesion synthesis (TLS) of DNA adducts that stall replication fork progression. Previous studies have led to the suggestion that the primary role of Pol ζ in TLS is to extend primers created when another DNA polymerase inserts nucleotides opposite lesions. Here we test the non-exclusive possibility that Pol ζ can sometimes perform TLS in the absence of any other polymerase. To do so, we quantified the efficiency with which *S. cerevisiae* Pol ζ bypasses abasic sites, *cis-syn* cyclobutane pyrimidine dimers and (6-4) photoproducts. In reactions containing dNTP concentrations that mimic those induced by DNA damage, a Pol ζ derivative with phenylalanine substituted for leucine 979 at the polymerase active site bypasses all three lesions at efficiencies between 27 and 73%. Wild-type Pol ζ also bypasses these lesions, with efficiencies that are lower and depend on the sequence context in which the lesion resides. The results are consistent with the hypothesis that, in addition to extending aberrant termini created by other DNA polymerases, Pol ζ has the potential to be the sole DNA polymerase involved in TLS.

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1. Introduction

When lesions in DNA impede DNA synthesis, the impediment can be alleviated by translesion DNA synthesis (TLS) catalyzed by specialized DNA polymerases. Included among several TLS polymerases is eukaryotic DNA polymerase ζ, an exonuclease-deficient, heterodimeric (Rev3-Rev7) DNA polymerase that participates in a variety of DNA transactions. These transactions contribute to spontaneous mutagenesis [1–4], mutagenesis induced by DNA damaging agents such as UV irradiation [5–8], mutagenesis associated with repair of double-strand DNA breaks [9–12], mutagenesis associated with high levels of transcription, cytosine deamination-dependent somatic hypermutation of immunoglobulin genes [13,14] and mutagenesis in cells defective in NER, BER, replication fork progression and post-replication repair [15–20]. That the cellular functions of Pol ζ are important is further revealed by the embryonic lethality resulting from loss of the mouse *REV3L* gene [21–23], the increased cancer susceptibility observed in a conditional knockout mouse model [24], and the increased cisplatin sensitivity of lung tumors in mice upon suppression of Rev3 [25].

Many of the phenotypes observed in genetic studies of Pol ζ are thought to reflect the ability of Pol ζ to participate in TLS, a subject that has been extensively investigated (reviewed by [1,26,27]). Several biochemical studies have reported that Pol ζ alone cannot efficiently bypass a UV-induced *cis-syn* cyclobutane pyrimidine dimer (CPD) [28,29], a (6-4) photoproduct [28–30] or an abasic site [29,31]. This limitation has been ascribed to inefficient insertion of a nucleotide opposite the first base of the dipyrimidine lesions or opposite the abasic site. However, Pol ζ is highly efficient at extending aberrant primer-templates, especially those that already contain a nucleotide present opposite a lesion [29–34]. These observations have led to a now widespread view that the primary role of Pol ζ in TLS is to extend primer-templates after a nucleotide has first been inserted opposite a lesion by another DNA polymerase. This is referred to as the two-polymerase model for TLS.

While there is substantial experimental support for the two-polymerase TLS model [1,26,27,35–37], an additional and non-exclusive possibility is that Pol ζ is sometimes the sole TLS polymerase involved in lesion bypass. This possibility is supported by several observations. In their seminal description of the discovery of yeast Pol ζ, Nelson et al. [38] reported that Pol ζ could bypass a T-T *cis-syn* CPD ten-fold more efficiently than could the catalytic subunit of yeast Pol α. Yeast Pol ζ was later reported to also perform bypass of thymine glycol [39], limited bypass of a (6-4) photoproduct [28] and to bypass photoproducts generated by UV

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irradiation of a poly(dT)$_{29}$ template [40]. A study of the efficiency with which lesion-containing plasmids transform wild-type yeast strains versus strains deficient in different TLS polymerases led to the suggestion that Pol ζ is responsible not only for extension, but also for insertion opposite lesions, at least for bypass events other than those in which Pol η participates [41].

That Pol ζ might be the sole TLS polymerase involved in UV photoproduct bypass *in vivo* is further suggested by a genetic study utilizing a variant of yeast Pol ζ containing a phenylalanine substituted for leucine 979, a conserved residue at the active site in the catalytic Rev3 subunit of the Rev3-Rev7 heterodimeric polymerase. A yeast strain harboring the *rev3-L979F* allele has wild-type survival following UV irradiation [42], consistent with the fact that purified L979F Pol ζ has robust polymerase activity [43], as needed to enhance survival following UV irradiation [5–8]. The *rev3-L979F* strain also has an elevated UV-induced mutation frequency compared to the wild-type *REV3* strain [42], consistent with the fact that L979F Pol ζ (L979F Rev3-Rev7) has lower fidelity than wild-type Pol ζ during DNA synthesis *in vitro* [43]. This indicates that, like wild-type Pol ζ, L979F Pol ζ also participates in mutagenic bypass of UV photoproducts *in vivo*. Moreover, UV-induced mutagenesis is further elevated when the *RAD30* gene encoding Pol η is deleted from the *rev3-L979F* strain [42]. This demonstrates that L979F Pol ζ contributes to bypassing UV photoproducts *in vivo* even in the absence of Pol η, the major yeast TLS polymerase implicated in insertion opposite lesions in the two-polymerase TLS model. Thus, either a polymerase other than Pol η or Pol ζ performs the initial insertion, or yeast Pol ζ alone can perform TLS *in vivo*.

The observations discussed above raise the issue of how efficiently yeast L979F Pol ζ and wild-type yeast Pol ζ perform TLS without assistance from other DNA polymerases. Here we examine this issue by performing biochemical studies of yeast Pol ζ lesion bypass that are similar to earlier TLS studies yet take into account three additional parameters. Many studies have examined TLS using amounts of polymerase and incubation times that result in a large, but often unknown, number of cycles of polymerase binding, synthesis and termination, which Nelson et al. [38] called "forcing conditions". When bypass is observed under such conditions, it is not possible to quantify bypass efficiency per synthesis cycle, making it difficult to compare TLS efficiency from one study to another. Here, as in several of our earlier TLS studies [44–47], we determine the relative bypass efficiency of Pol ζ per cycle of polymerization, thus permitting direct comparisons to other polymerases when analyzed in the same manner [44]. We also take into account the fact that the concentrations of the four dNTPs are not equal *in vivo* and that yeast cells respond to exposure to DNA damaging agents by up-regulating dNTP pools [48,49]. As a consequence, yeast TLS polymerases may perform bypass *in vivo* using dNTP concentrations that are unequal and that may be higher than those used previously for bypass studies *in vitro*. Unequal dNTP concentrations could influence bypass efficiency in a sequence-dependent manner. High, damage-induced dNTP concentrations can also increase TLS efficiency, as evidenced by the increased efficiency with which DNA Pol ε bypasses 8-oxo-guanine at high dNTP concentrations [47], and the increased efficiency with which DNA polymerases δ and ε bypass rNMPs in template DNA at high dNTP concentrations [50].

Here we examine the ability of Pol ζ to bypass lesions using dNTP concentrations approximating those induced upon exposure of yeast to UV light [49] or chronic exposure to 4-NQO [48], which has frequently been used as a UV mimetic. The results show that, without assistance from other DNA polymerases, L979F Pol ζ can bypass synthetic abasic sites, T-T *cis-syn* CPDs and T-T (6-4) photoproducts in two different sequence contexts. Bypass per cycle of polymerization is remarkably efficient, supporting a model wherein L979F Pol ζ is the only polymerase needed for UV photoproduct bypass in the *rev3-L979F rad30Δ* strain. The results show that wild-type Pol ζ can also bypass these lesions, albeit with lower efficiencies that vary depending on the lesion and the DNA sequence in which it is embedded. These data imply that, in addition to a prominent role in extending aberrant primers in a two-polymerase model, Pol ζ has the biochemical potential to function as the sole polymerase involved in TLS.

2. Materials and methods

2.1. Measurements of dNTP pools and cell cycle progression in yeast strains

The previously described wild-type and *rev3-L979F S. cerevisiae* strains [42] were grown in YPDA media (1% yeast extract, 2% bacto-peptone 2% dextrose and, 250 mg/l adenine). Measurements of dNTP levels in extracts prepared from asynchronous, logarithmically growing yeast cells were performed by HPLC analysis as described [51]. Flow cytometry was performed as previously described [47].

2.2. Protein purification

S. cerevisiae Pol ζ (Rev3-Rev7) and L979F Pol ζ (L979F Rev3-Rev7) were over-expressed and purified from yeast as previously described [40]. SDS-PAGE gels of purified two-subunit wild-type and L979F Pol ζ were shown previously [43]. *S. cerevisiae* Pol δ was purified as previously described [52].

2.3. DNA substrates

All substrates are listed in Table 1. Substrates were prepared by annealing ^{32}P-labelled primer oligonucleotides and template oligonucleotides in a 1:1.5 ratio, as described previously [45].

2.4. Lesion bypass assay

Wild-type and L979F Pol ζ reactions (30 μl) contained 40 mM Tris–HCL (pH 7.8), 100 mM NaCl, 20 mg/ml BSA, 8 mM MgAc, 1 pmol DNA substrate, 120 fmol Pol ζ or L979F Pol ζ and dNTPs. The dNTP concentrations were either 16 μM dATP, 30 μM dTTP, 14 μM dCTP, and 12 μM dGTP (referred to as "normal"), or 10-fold higher concentrations of each dNTP (refered to as "high"). Pol δ reactions (30 μl) contained 20 mM Tris–HCL (pH 7.8), 90 mM NaCl, 200 mg/ml BSA, 1 mM DTT, 8 mM MgAc, 160 μM dATP, 300 μM dTTP, 140 μM dCTP, and 120 μM dGTP, 1 pmol DNA substrate and the indicated amount of polymerase. Reactions were performed in duplicate. All components except polymerase were mixed on ice and incubated at 30 °C for 2 min. Polymerase was added to start the reactions, which were incubated at 30 °C. Aliquots were removed at the times specified and added to an equal volume of formamide loading buffer (95% deionized formamide, 25 mM EDTA, 0.01% bromophenol blue and 0.01% xylene cyanol) to stop the reaction. DNA products were separated by electrophoresis using 12% denaturing polyacrylamide gels and quantified using ImageQuant version 5.2 (Molecular Dynamics). As previously described [53,54], bypass probability is defined as the summed intensity of all product bands corresponding to synthesis after the lesion (*e.g.* in Fig. 2, positions +1 and greater) divided by the summed intensity of all bands corresponding to synthesis before the lesion is encountered (*e.g.* in Fig. 2, positions −1 and −2). Termination probability of a particular template position is calculated dividing the intensity of the band that corresponds to that position by the sum of the intensity of that position plus the intensity

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