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The choice of nucleotide inserted opposite abasic sites formed within chromosomal DNA reveals the polymerase activities participating in translesion DNA synthesis

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ABSTRACT

Abasic sites in genomic DNA can be a significant source of mutagenesis in biological systems, including human cancers. Such mutagenesis requires translesion DNA synthesis (TLS) bypass of the abasic site by specialized DNA polymerases. The abasic site bypass specificity of TLS proteins had been studied by multiple means *in vivo* and *in vitro*, although the generality of the conclusions reached have been uncertain. Here, we introduce a set of yeast reporter strains for investigating the *in vivo* specificity of abasic site bypass at numerous random positions within chromosomal DNA. When shifted to 37 °C, these strains underwent telomere uncapping and resection that exposed reporter genes within a long 3′ ssDNA overhang. Human APOBEC3G cytosine deaminase was expressed to create uracils in ssDNA, which were excised by uracil-DNA N-glycosylase. During repair synthesis, error-prone TLS bypassed the resulting abasic sites. Because of APOBEC3G's strict motif specificity and the restriction of abasic site formation to only one DNA strand, this system provides complete information about the location of abasic sites that led to mutations. We recapitulated previous findings on the roles of *REV1* and *REV3*. Further, we found that sequence context can strongly influence the relative frequency of A or C insertion. We also found that deletion of Pol32, a non-essential common subunit of Pols δ and ζ, resulted in residual low-frequency C insertion dependent on Rev1 catalysis. We summarize our results in a detailed model of the interplay between TLS components leading to error-prone bypass of abasic sites. Our results underscore the utility of this system for studying TLS bypass of many types of lesions within genomic DNA.

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1. Introduction

DNA is under constant threat of damage by both endogenous and environmental agents [1]. Damage to the nitrogenous bases that result in miscoding can be particularly deleterious if left unrepaired before a subsequent round of replication, as this can result in mutation fixation [1]. A principal means of correcting various adduct lesions is by base excision repair (BER), which is initiated when a DNA N-glycosylase specifically recognizes its cognate adducted base substrate and excises it to generate an abasic site [2]. Abasic sites also are generated when glycosylases erroneously excise normal bases [3] or by spontaneous breakage of the N-glycosidic bond, especially in adducted nucleotides where this bond is destabilized [4]. Usually, short- or long-patch BER replaces the damaged nucleotide (along with some neighboring residues, in the latter case), using the intact complementary strand as a template [2].

Abasic sites are thought to be one of the most common lesions within DNA, under steady state, unstressed conditions [5]. While one would expect that the majority of such lesions should be repaired correctly by BER, abasic sites formed within single-strand DNA (ssDNA) likely would not be subject to such error-free repair. This is because the complementary strand to template repair synthesis is, by definition, absent. Moreover, it would be counterproductive to attempt BER on an abasic site within ssDNA of a replication fork, as doing so would risk strand breakage and fork collapse [6]. Similarly, BER would be unavailable for repairing abasic sites formed by strand resection from double-strand breaks or within subtelomeric ssDNA following telomere uncapping. In the latter scenario, we previously observed clusters of multiple base substitutions originating from abasic sites on the 3′ ssDNA overhang strand [7]. More recently, Neuberger and colleagues expressed hyperactive cytosine deaminases in yeast and found similar mutation showers, which were associated with breakpoints and thought to result from damage to ssDNA at resected double strand

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Table 1
List of yeast strains used in this study.

Strain name(s)	Genotype	Plasmid
yKC066, yKC067	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)[a]	pCM252-A3G
yKC068, yKC069	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)[a]	pCM252
yKC213, yKC214	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC217, yKC218	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC131, yKC135	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev3::NatR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)[a]	pCM252-A3G
yKC133, yKC137	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev3::NatR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)[a]	pCM252
yKC205, yKC206	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev1::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC209, yKC210	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev1::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC385	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev1-AA lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC387, yKC388	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rev1-AA lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC299, yKC230	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::HygR rev3::NatR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC233, yKC234	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::HygR rev3::NatR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC245, yKC246	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::KanMX4 rev1::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC249, yKC250	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 rad30::KanMX4 rev1::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC261, yKC262	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 pol32::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC265	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 pol32::HygR lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252
yKC399	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 pol32::HygR rev1-AA lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252-A3G
yKC400, yKC402	*MATα his7-2 leu2-3,112 trp1-289 cdc13-1 pol32::HygR rev1-AA lys2::CAN1-URA3-ADE2* (in sub-telomere 5L)	pCM252

[a] The WT and *rev3* strains were described previously in [7]. All other yeast strains originate from this study.

breaks [8]. Such formations as observed by both groups are reminiscent of similar clusters of multiple point mutations identified among various human cancers, also likely to have originated from many abasic sites within long stretches of ssDNA [9–11].

When confronted with abasic sites in a DNA configuration that is not amenable to BER, the cell resorts to using mechanisms of DNA damage tolerance that result in bypass of the lesion without repairing it (reviewed recently in [6,12]). One such mechanism is translesion DNA synthesis (TLS) by specialized DNA polymerases, which can be error-prone, *i.e.* mutagenic (reviewed recently in [12–14]). This is because TLS polymerases can contain relatively large active sites that accommodate non-canonical bases (*e.g.*, [15,16]) and lack the proofreading activity of the higher fidelity replicative polymerases (reviewed in [17,18]). As a result, TLS polymerases are considerably more error-prone, even when copying undamaged DNA templates (*e.g.*, [19,20]). Thus, cells have evolved these low fidelity polymerases as a means to bypass genomic lesions that would otherwise block replication, but at the cost of possible mutation fixation [12–14].

Conserved proteins that take part in TLS have been identified in many organisms, including budding yeast [21–24]. Yeast has a repertoire of three specialized TLS polymerases whose functional specificity in bypassing abasic sites has been investigated by the following approaches: *in vitro* TLS assays (*e.g.*, [25–27]); transfecting cells with plasmids carrying a single, engineered abasic site [28–30]; expressing mutator DNA glycosylases that generate excess abasic sites in BER-deficient strains [31,32]; and analyzing unselected abasic site-associated TLS events within a frameshift reversion reporter, in excision repair defective backgrounds [33]. Pol η, which catalyzes error-free bypass of UV-induced cyclobutane pyrimidine dimers, is encoded by the *RAD30* gene [34]. Pol ζ is proficient at extending from various mismatched termini during TLS *in vitro* (*e.g.*, [27,35,36]) and includes a catalytic subunit encoded by *REV3* along with an accessory subunit encoded by *REV7* [37]. There is also evidence that Pol ζ, acting alone, can bypass some lesions *in vitro* [37,38]. *REV1* encodes a protein with deoxycytidyltransferase activity [26] and provides a structural function crucial for TLS bypass of certain lesions (*e.g.*, [39–41]). Additionally, the replicative polymerase δ has been implicated in the insertion of A opposite abasic sites [27]. A non-essential accessory subunit of both Pol δ and Pol ζ, encoded by *POL32* [42–44], is necessary for efficient TLS bypass of various lesions, including abasic sites [29,30].

Previously, we used a subtelomeric ssDNA mutagenesis reporter system to confirm that the mutagenic bypass of abasic sites (resulting from Ung1-catalyzed excision of uracils [45] that were formed by cytosine deamination) within chromosomal DNA required the TLS activity of Pol ζ [7]. In contrast, when uracils were left intact in *ung1Δ* cells, mutagenesis was completely independent of TLS, as deletion of *REV3* did not affect the frequency of gene inactivation at all in this background [7]. Thus, the TLS dependence in cells with Ung1 argues that mutagenesis due to residual amounts of unexcised uracils was at most, a minor factor. Here, we apply the subtelomeric ssDNA mutagenesis reporter system to investigate the roles of TLS proteins in the error-prone bypass of randomly generated abasic sites within chromosomal DNA. We found that similar to human cancers [9–11], A and C tend to be incorporated opposite abasic sites at similar frequencies. Based on our results, we infer the respective roles and relative contributions of the various TLS proteins toward the error-prone bypass of abasic sites within genomic DNA.

2. Materials and methods

2.1. Yeast strains

Yeast strains used in this study are listed in Table 1. All are isogenic to CG379 [46] with the following common markers: *MATα his7-2 leu2-3,112 trp1-289*. WT and *rev3* subtelomeric reporter strains bearing tetracycline regulatable APOBEC3G plasmids were described previously [7]. Other TLS gene deletion strains were constructed by one step gene replacement [47]. The *rev1-AA* allele (with D647A and E648A amino acid substitutions [27]) was constructed by the *delitto perfetto* approach [48]. Strain constructions were verified by diagnostic replica plating, PCR, and sequencing (for *rev1-AA*). Strains were maintained on TRP dropout media to select for plasmid retention.

2.2. Determination of CAN1 inactivation frequency

For each combination of genotype and plasmid, multiple independent colonies each were inoculated into 5 mL of YPDA liquid (1% yeast extract, 2% peptone, 2% dextrose, supplemented with 0.01% adenine sulfate, filter-sterilized) and grown at 23 °C for 2 days. Then, 500 μL of each culture was added to 4.5 mL of fresh YPDA with 10 μg/mL doxycycline hyclate [49] (Sigma–Aldrich, St. Louis, MO) and shifted to 37 °C for 6 h. Cells were counted and G_2 arrest was monitored by examining cell morphology. Cells were washed once in water. 500 cells were plated, in triplicate, onto synthetic complete to determine plating efficiency. An appropriate dilution