Evidence for lesion bypass by yeast replicative DNA polymerases during DNA damage

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ABSTRACT

The enzyme ribonucleotide reductase, responsible for the synthesis of deoxyribonucleotides (dNTP), is upregulated in response to DNA damage in all organisms. In Saccharomyces cerevisiae, dNTP concentration increases 6- to 8-fold in response to DNA damage. This concentration increase is associated with improved tolerance of DNA damage, suggesting that translesion DNA synthesis is more efficient at elevated dNTP concentration. Here we show that in a yeast strain with all specialized translesion DNA polymerases deleted, 4-nitroquinoline oxide (4-NQO) treatment increases mutation frequency ~3-fold, and that an increase in dNTP concentration significantly improves the tolerance of this strain to 4-NQO induced damage. In vitro, under single-hit conditions, the replicative DNA polymerase ε does not bypass 7,8-dihydro-8-oxoguanine lesion (8-oxoG, one of the lesions produced by 4-NQO) at S-phase dNTP concentration, but does bypass the same lesion with 19–27% efficiency at DNA-damage-state dNTP concentration. The nucleotide inserted opposite 8-oxoG is dATP. We propose that during DNA damage in S. cerevisiae increased dNTP concentration allows replicative DNA polymerases to bypass certain DNA lesions.

INTRODUCTION

Ribonucleotide reductases (RNRs) catalyze the formation of dNTPs by reducing the corresponding ribonucleotides, and are instrumental in controlling dNTP concentration (1). In eukaryotes and in some bacteria, RNR is composed of a large and a small subunit, both necessary for catalysis. RNR expression increases in response to DNA damage. In Escherichia coli, nrdA and nrdB (encoding the large and the small RNR subunits, respectively) are among the most potently induced lexA-independent genes following UV exposure (induced ~20- and ~7-fold, respectively, within 60 min of UV exposure) (2,3). In resting mammalian cells, DNA damage induces the p53R2 protein, an alternative small RNR subunit, about 4-fold in a p53-dependent manner (4–6). Similarly, Drosophila large RNR subunit, RnrL, is induced by ionizing radiation in wild-type, but not p53-deficient strains (7). In the yeasts S. cerevisiae and S. pombe, RNR genes are also among the most robustly induced genes following DNA damage (8–10). In addition to transcriptional regulation, RNR activity in both yeasts is controlled by Sml1 and Spd1, small proteins that bind to RNR and inhibit its activity (11–13). Sml1 and Spd1 are degraded upon entry into S phase and in response to DNA damage (12,14). In S. cerevisiae, the large subunit is encoded under normal growth conditions by the RNR1 gene. During DNA damage, the highly similar RNR3 gene is activated, which leads to increased levels of the large subunit (8). The small subunit, responsible for generation of the free tyrosyl radical important for catalysis, is a heterodimer encoded by the RNR2 and RNR4 genes (15). The Mec1/Rad53 DNA damage checkpoint is responsible both for activation of RNR2-4 genes transcription and Sml1 degradation (14,16).

RNR activity is also controlled allosterically. The enzyme’s allosteric specificity sites, located in the large subunit, adjust the balance between the four individual dNTPs. The allosteric activity sites, also located in the large subunit control the overall concentration of dNTP: when the concentration of dNTP reaches a certain level, RNR activity is down-regulated by dATP feedback inhibition (17). Saccharomyces cerevisiae RNR has a relaxed dATP feedback inhibition, which allows at least a 6- to 8-fold increase of dNTP concentration in response to DNA damage, or at least an ~3- to 5-fold increase above the dNTP concentration of an S-phase yeast cell (18). This increase in dNTP concentration correlates directly to DNA damage tolerance. In the rnr1-D57N mutant strain, in which the dATP feedback inhibition of RNR is non-functional, dNTP concentration increases ~30-fold in response to DNA damage, ~4 times more...
than in a wild-type strain under similar conditions. The ability of the rnr1-D57N mutant to increase dNTP concentration above wild-type levels in response to DNA damage is associated with higher tolerance of DNA damage induced by 4-NQO, methyl methane sulfonate (MMS) and UV-light (18). 4-NQO produces several types of quinoline adducts at guanine and adenine bases as well as 8-oxoG (19). Overexpression of the wild-type RNR1 gene in logarithmically growing yeast elevates dNTP concentration ~10-fold and similarly leads to an increased DNA damage tolerance to 4-NQO (20). Deletion of Crt1/Rfx1, Rox1 or Mot3, transcriptional repressors of RNR2, RNR3 and RNR4 genes, also leads to 4-NQO resistance (21).

The improved DNA damage tolerance of S. cerevisiae in the presence of high dNTP concentration is associated with higher mutation frequency (18), and can be best explained by a more efficient translesion DNA synthesis (TLS). The specialized TLS polymerases Rev1, Polɛ and Polη are believed to be responsible for the mutagenic bypass of DNA lesions and increased damage tolerance. To identify translesion polymerases that increase DNA damage tolerance in the dNTP concentration-dependent manner, we made deletions of REV1, RAD30 (Polɛ), REV3 (the catalytic subunit of Polɛ), and POL4 (non-replicative DNA polymerase involved in DNA repair), and compared DNA damage tolerance of these deletion strains towards 4-NQO in the presence of normal and high dNTP concentrations. Deletion of REV1 or REV3, but not of RAD30 or POL4 resulted in sensitivity to 4-NQO. Interestingly, increased dNTP concentration significantly improved the 4-NQO tolerance in all TLS polymerase-deleted strains, including a strain with all non-replicative polymerases deleted. Mutation frequency in this strain increased ~3-fold after treatment with 4-NQO. These observations indicate that replicative DNA polymerases are able to bypass certain DNA lesions when dNTP concentration is elevated after DNA damage. In support of this hypothesis we show that in vitro, under single-hit conditions, the replicative DNA polymerase ε (Polɛ) does not bypass 8-oxoG lesion at S-phase dNTP concentration, but does bypass the same lesion with 19–27% efficiency at DNA-damage-state dNTP concentration.

MATERIALS AND METHODS

Yeast strains

All yeast strains are derivatives of W4069-4C (MATα CAN1 ade2-1 his3-11,15 leu2-3,112 trpl-1) (18) used as wild type and were grown in YP media (1% yeast extract, 2% peptone) with 2% dextrose (YPD) or 2% galactose (YPGαL). Construction of the pGAL-RNR1 strain was described before (20). TLS polymerase genes were deleted using cassettes polymerase chain reaction (PCR)-amplified from pFA6a-HIS3MX6 (for rev1Δ), pFA6a-kanMX6 (for rev1Δ, rev3Δ, rad30Δ) and pFA6a-TRP1(for pol4Δ) as previously described (22). REV3 was also deleted with LEU2 using pAM56 plasmid (23) kindly provided by Dr Alan Morrison. All deletion strains were back-crossed to wild-type and the correct insertion of a deletion cassette was confirmed by PCR. Construction of the rev1Δ::HIS3 rev3Δ::Leu2 polyΔ::TRP1 strain was done by crossing single TLS polymerase deletion strains with each other. Introduction of the pGAL-RNR1 into different strains was also done by crossing.

dNTP analysis

At a density from 0.5 × 10^7 to 1.5 × 10^7 cells/ml, ~1 × 10^6 cells were harvested by filtration through 25 mm White AAWP nitrocellulose filters (0.8 μm, Millipore AB, Solna, Sweden). The filters were immersed in 500 μl of ice-cold extraction solution (12% w/v trichloroacetic acid, 15 mM MgCl2) in Eppendorf tubes. The following steps were carried out at 4°C. The tubes were vortexed for 30 s, incubated for 15 min and vortexed again for 30 s. The filters were removed and the supernatants were collected after centrifugation at 20000g for 1 min and added to 800 μl of ice-cold Freon-trioctylamine mixture [10 ml of Freon (1,1,2-trichlorotrifluoroethane, Aldrich, Sigma-Aldrich Sweden AB, Stockholm, Sweden, 99%) and 2.8 ml of trioctylamine (Fluka, Sigma-Aldrich Sweden AB, Stockholm, Sweden, >99%)]. The samples were vortexed and centrifuged for 1 min at 20 000g. The aqueous phase was collected and added to 800 μl of ice-cold Freon-trioctylamine mixture. The mixture was vortexed and centrifuged as above. Twenty microliters of the aqueous phase containing dNTP and NTP was analyzed by HPLC on a Partisil SAX column (PolyLC Inc., Columbia, MD, USA) using a UV-2075 Plus detector (Jasco, Mölndal, Sweden). Nucleotides were isocratically eluted with 2.5% acetonitrile, 0.3 M potassium phosphate, pH 5.0 buffer.

Flow cytometry

At the density 0.5 × 10^7 to 1.5 × 10^7 cells/ml, ~1 × 10^6 cells were harvested by filtration through 25 mm White AAWP nitrocellulose filters (0.8 μm, Millipore). The filters were immersed into 13 ml tubes with 1.5 ml H2O and vortexed to wash the cells off the filters. Total 3.5 ml of 99% ethanol was added dropwise with slow vortexing and cells were kept at 4°C overnight. The filters were removed; the cells were collected by centrifugation, resuspended in 700 μl of H2O, transferred to Eppendorf tubes and centrifuged. The cells were resuspended in RNase solution (2 mg/ml RNase in 50 mM Tris pH 8.0, boiled 15 min) and incubated 6–15 h at 37°C. Fifty microliters of 20 mg/ml proteinase K in H2O was added and the cells were incubated 1 h at 50°C. The cells were collected by centrifugation, resuspended in 0.5 ml 50 mM Tris pH 7.5. For analysis, 50 μl of cell suspension was placed into 1 ml of staining solution (SYBR®-Green I (Molecular Probes) diluted 10 000 times in 50 mM Tris, pH 7.5). Samples were sonicated at low output and analyzed on a Cytomics FC500 (Beckman Coulter Inc, Bromma, Sweden).

Primer extension assay

Polɛ was purified as described (24). Primer extension assays were performed as described (25), but with varying dNTP concentrations as indicated in Table 1 and with the
Table 1. dNTP concentrations used in primer extension assays shown in Figure 4

| Cell volume (µm³) | dNTP concentration | dNTP (µM) |
|------------------|---------------------|-----------|
|                  | dCTP | dTPP | dATP | dGTP |
| 90               | Low  | 10   | 16   | 5.5  | 3    |
|                  | Normal | 19.5 | 33   | 11   | 5.5  |
|                  | High  | 97.5 | 191.5 | 97   | 25   |
| 45               | Low  | 19.5 | 33   | 11   | 5.5  |
|                  | Normal | 39   | 66   | 22   | 11   |
|                  | High  | 195  | 383  | 194  | 49.5 |

‘Normal’ is an estimated S-phase cell dNTP concentration; ‘Low’ is half of ‘Normal’ and is approximately an average concentration of a logarithmically growing yeast culture; ‘High’ is an approximated maximal dNTP concentration of a DNA-damaged cell (dCTP is 5-fold, dTTP is 5.8-fold, dATP is 8.8-fold and dGTP is 4.5-fold above minimal dNTP concentration of a DNA-damaged cell (dCTP is 5-fold, dTTP is 5.8-fold, dATP is 8.8-fold and dGTP is 4.5-fold above ‘Normal’).

following primer (5'-CTGACAGTGAACCATTACACTGACGATCGATCGTCGCTAC-3') annealed to the wild-type, 8-oxoG or MeG templates (5'-GATCGATCGTAACzTAGCAGGATCGAATCGTCCTTAGAGGATACTATCGAATCGTGTTGATGGTATACGTACACTGTCG-3'), where z indicates a G, an 8-oxoG or MeG. The reaction mixtures were separated on an 8% denaturing polyacrylamide gel and visualized with a Typhoon 9400 PhosphorImager (GE Healthcare Biosciences, Uppsala, Sweden). The intensities of the bands were quantified using ImageQuant software package supplied with the PhosphorImager.

Analysis of base insertion opposite 8-oxoG

Biotinylated Acc65I overhang primer (5'-Biotin GTA GGATCCTAGATATCCCTAAGGACGATTCTGCGATCTG-3') was annealed to the wild-type, 8-oxoG or MeG templates (5'-GATCGATCGTAACzTAGCAGGATCGAATCGTCCTTAGAGGATACTATCGAATCGTGTTGATGGTATACGTACACTGTCG-3'), where z indicates a G, an 8-oxoG or MeG. The reaction mixtures were separated on an 8% denaturing polyacrylamide gel and visualized with a Typhoon 9400 PhosphorImager (GE Healthcare Biosciences, Uppsala, Sweden). The intensities of the bands were quantified using ImageQuant software package supplied with the PhosphorImager.

RESULTS

Overexpression of RNR1 efficiently elevates dNTP concentration

To establish strains, in which dNTP concentration could be experimentally controlled, we utilized the GAL1-driven wild-type RNR1 gene introduced into the URA3 locus of the yeast genome. We measured dNTP pools in the rev1Δ rad30Δ rev3Δ pol4Δ and rev1Δ rad30Δ rev3Δ pol4Δ pGAL-RNR1 strains grown in galactose-containing media before and after DNA damage induced by 4-NQO (Figure 1a). Induction of the RNR1 gene by galactose in the rev1Δ rad30Δ rev3Δ pol4Δ pGAL-RNR1 strain resulted in overexpression of the Rnr1 protein and a 9- to 13-fold elevation of dNTP concentration compared to rev1Δ rad30Δ rev3Δ pol4Δ strain (Figure 1b and c). Addition of 4-NQO to the rev1Δ rad30Δ rev3Δ pol4Δ pGAL-RNR1 strain induced by galactose further increased dNTP concentration 3- to 4-fold (Figure 1b). This further increase can be explained by the induction of the RNR2-4 genes, degradation of Sml1 and a decreased utilization of dNTP during DNA damage.

DNA damage tolerance of the TLS polymerase deletion strains increases in the presence of elevated dNTP concentration

If a certain TLS polymerase were responsible for the bypass of a 4-NQO lesion only at a high dNTP concentration, then deletion of this polymerase would result in a yeast strain equally sensitive to 4-NQO at normal and high dNTP concentrations. In all single polymerase deletion strains the elevation of dNTP concentration improved DNA damage tolerance (survival of DNA damage) (Figure 2a). Deletion of REV1 or REV3 resulted in sensitivity to 4-NQO, while the rad30Δ and pol4Δ strains were not 4-NQO sensitive (Figure 2a). Next, we tested
the DNA damage tolerance of a strain with all non-replicative nuclear polymerases deleted (rev1Δ rad30Δ rev3Δ pol4Δ), with or without RNR1 overexpression. Strikingly, overexpression of RNR1 (resulting in a 3- to 4-fold higher dNTP concentration under these conditions, compare samples 2 and 4 in Figure 1b) improved the DNA damage tolerance of the rev1Δ rad30Δ rev3Δ pol4Δ strain to 4-NQO up to 100-fold (Figure 2b and Supplementary Figure 1). The elevation of dNTP concentration also improved the tolerance to 4-NQO in a wild-type strain with all polymerases present, as observed earlier (18, 21).

**4-NQO increases the mutation frequency 3-fold in a rev1Δ rad30Δ rev3Δ pol4Δ strain**

The increased DNA damage tolerance of the rev1Δ rad30Δ rev3Δ pol4Δ strain in the presence of elevated dNTP concentration suggests that the replicative DNA polymerases are able to bypass some lesions produced by 4-NQO. Alternatively, other DNA repair pathways, e.g. nucleotide excision repair (NER) or base excision repair (BER), are somehow stimulated by increased dNTP pools. However, these pathways do not involve a direct bypass of a lesion by a DNA polymerase and should not be mutagenic. Therefore, we measured the induced mutation frequencies in the rev1Δ rad30Δ rev3Δ pol4Δ and wild-type strains after 2 h incubation with increasing concentrations of 4-NQO. The initial increase in the induced mutation frequencies (about 3-fold) and the initial decrease in survival showed the same dynamics in both strains (Figure 3a and b). At 0.04 mg/l 4-NQO the induced mutation frequency in the rev1Δ rad30Δ rev3Δ pol4Δ strain reached a plateau, while the induced mutation frequency in the wild-type strain continued to increase. Since in both strains the treatment with 4-NQO leads to...
elevation of dNTP pools ∼8-fold (Figure 1b), it is possible that the observed initial increase in mutation frequencies is due to higher error rates of replicative polymerases in the presence of high dNTP concentration and not due to lesion bypass. However, mutation frequencies did not increase in the rev1A rad30Δ rev3Δ pol4Δ pGAL-RNRI strain induced by galactose for 2 or 4 h (Figure 3c), even though the dNTP concentration increases ∼10-fold after the galactose induction in the absence of 4-NQO (Figure 1b). Thus, the increase in the 4-NQO-induced mutation frequency in the rev1A rad30Δ rev3Δ pol4Δ strain is most likely due to increased translesion synthesis by the replicative DNA polymerases.

**Bypass of 8-oxoG by Polε at S-phase and DNA-damage-state dNTP concentrations**

4-NQO produces several types of quinoline adducts to guanine and adenine bases as well as a common DNA lesion, 8-oxoG (19). The ratio between the quinoline-bound adducts and the 8-oxoG found in the DNA of Ehrlich ascites cells exposed to 4-NQO was estimated to be 4:1 (26). We assessed the ability of yeast replicative Polε to bypass 8-oxoG *in vitro* at the dNTP concentration found *in vivo* in wild-type cells during a normal S phase and during DNA damage. Polε is one of the three replicative yeast DNA polymerases and, together with Polα, is responsible for the bulk of DNA synthesis (27, 28). The intracellular dNTP concentrations were calculated using the published amount of dNTP per million of wild-type haploid yeast cells grown in YPD (11, 18), and the reported wet (60 × 10⁻¹² g) and dry (15 × 10⁻¹² g) weight of a haploid yeast cell (29). Next, the dry weight was subtracted from the wet weight to estimate the volume of the soluble fraction of a haploid cell (45 × 10⁻¹² g or ∼45 μm³). Because yeast cells increase in volume during the cell cycle arrest elicited by DNA damage, and because in some reports the volume of yeast cells is 70 μm³ and greater, we calculated dNTP concentration using two volumes: 45 and 90 μm³ (Table 1).

The ability of the wild-type, proofreading-proficient, Polε to bypass an 8-oxoG lesion increased dramatically at an elevated dNTP concentration approximating the DNA-damaged-state concentration (Table 1, ‘High’) as compared to S-phase dNTP concentration (Table 1, ‘Normal’). In the presence of excess Polα; over template, the 8-oxoG lesion bypass increased from 19% at S-phase dNTP concentration to 93% at DNA-damaged-state dNTP concentration for a 45 μm³ cell (Figure 4a, compare lanes 12 and 13), or from 8% to 66% for a 90 μm³ cell (Figure 4a, compare lanes 6 and 7). Under single-hit conditions, when the reactions were performed with an excess of template over Polε to ensure that each product was from only one replication event, we observed no 8-oxoG bypass at low (Table 1, ‘Low’ and Figure 4b, lanes 5 and 11) or S-phase dNTP concentrations (Table 1, ‘Normal’ and Figure 4b, lanes 6 and 12), but 16 and 25% bypass probability at DNA-damaged-state dNTP concentrations for the 90 and 45 μm³ cell, respectively (Table 1, ‘High’ and Figure 4b, lanes 7 and 13). To calculate the bypass efficiency we divided the bypass probability of the damaged template with the bypass probability of the undamaged template (30). The bypass efficiency at ‘Low’ and S-phase dNTP concentrations was 0%, and at DNA-damaged-state dNTP concentrations 19 and 27%, for the 90 and 45 μm³ cell, respectively. Therefore, approximately 20% of the time, Polε bypasses an 8-oxoG lesion at DNA-damaged-state dNTP concentration without dissociating from the template. We have identified dAMP as the major nucleotide inserted by Polε opposite 8-oxoG (Table 2) at dNTP concentrations present *in vivo* after DNA damage. Insertion of dAMP opposite 8-oxoG has also been observed for Polδ, although in the presence of equimolar dNTP concentrations (31).
Another common lesion, O\(^6\)-Methylguanine (MeG) is also bypassed by Pol\(e\) under single-hit conditions at DNA-damaged-state dNTP concentrations, but not at S-phase dNTP concentrations (Figure 4c and d). The bypass efficiency at S-phase dNTP concentrations was 0% and at DNA-damage-state dNTP concentrations 10 and 22%, for the 90 and 45\(\mu\)m\(^3\) cell, respectively.

**DISCUSSION**

Until the discovery of specialized TLS polymerases, it was generally believed that DNA lesions were bypassed by replicative DNA polymerases. In the last decade the focus has almost entirely shifted to investigation of DNA lesion bypass by TLS polymerases, while the role of replicative polymerases has received much less attention. Here, we address the involvement of yeast replicative and TLS DNA polymerases in the bypass of DNA lesions at normal and elevated dNTP concentrations.

In *S. cerevisiae*, the dNTP concentration increases 6- to 8-fold in response to DNA damage (18). The dNTP concentration increase is noticeable already at very low concentrations of mutagens (e.g. 0.01 mg/l NQO), long before cell proliferation is affected. An artificial elevation of dNTP concentration above normal levels in an *rnr1* mutant strain results in increased DNA damage tolerance.
and higher mutation rates. It is important to note that the DNA damage checkpoint is not pre-activated by an artificial elevation of dNTP concentration (20), and thus cannot be accounted for higher DNA damage tolerance. Conversely, a decrease in RNR activity by deletion of the RNR4 gene leads to a smaller increase of dNTP concentration after DNA damage, lower induced mutation frequencies and higher DNA damage sensitivity (32–34). These observations indicate that an increase in dNTP concentration is important for the ability of DNA polymerases to bypass DNA lesions. Here, we demonstrate that an artificial increase in dNTP concentration in a strain with all non-replicative polymerases deleted increases 4-NQO tolerance up to 100-fold. Furthermore, we demonstrate that 4-NQO elevates mutation frequency ~3-fold in a strain with all non-replicative polymerases deleted. This increase is not due to higher mutation rates of replicative DNA polymerases in the presence of elevated dNTP under these conditions, as an artificial increase in dNTP concentration (to the same levels as in the 4-NQO-treated yeast) in the absence of DNA damage does not elevate the mutation frequency. These observations indicate that replicative DNA polymerases can bypass certain DNA lesions in the presence of increased dNTP concentration. It should be, however, noted that the involvement of replicative polymerases in lesion bypass in vivo has only been demonstrated when all non-replicative polymerases have been deleted, and that the involvement of replicative polymerases in TLS in normal cells has not yet been proven.

Yeast replicative DNA polymerases a and δ (mainly involved in the lagging strand synthesis) are able to insert nucleotides opposite DNA lesions when dNTP concentration is sufficiently high (35,36). For example, Polδ is able to partially bypass an 8-oxoG lesion at 100 μM, but not at 5 μM dNTP (35). However, as the dNTP concentration in vivo under the DNA damaging conditions has not been defined, the physiological relevance of these observations was not obvious. Here we show for the first time that nucleotides can be inserted opposite 8-oxoG and MeG lesions by the yeast leading strand polymerase, Polε, at dNTP concentrations and at correct dNTP pool bias present in vivo during DNA damage. These data underscore the importance of elevated dNTP concentration for unaided lesion bypass by replicative DNA polymerases. In addition, the insertion of nucleotides by replicative polymerases opposite lesions can provide a substrate for the TLS Polζ. This TLS polymerase is inefficient at inserting nucleotides opposite various DNA lesions including 8-oxoG, but is efficient at extending from nucleotides inserted opposite these lesions by the replicative DNA Polδ (35,37).

It should be pointed out that 8-oxoG and MeG are examples demonstrating the ability of replicative polymerases to directly bypass certain lesions at dNTP concentrations present during DNA damage in vivo. It might be, however, some other lesions and not necessarily the 8-oxoG lesion that are bypassed by the replicative polymerases in the experiments with 4-NQO and elevated dNTP concentration. We were not able to test the ability of Polδ to bypass other known lesions induced by 4-NQO, e.g. those containing guanine bases with quinoline adducts, because of the difficulty to synthesize such templates. It is, however, less likely that Polε would be able to bypass such bulky lesions as quinoline adducts efficiently, considering that Polε can not bypass thymine dimer or (+)- and (-)-trans-anti-benz[a]pyrene-7,8-dihydriodiol-9,10-epoxide-N2-dG DNA adducts (38,39). The inability of replicative polymerases to bypass bulky lesions even at very high dNTP concentration is in agreement with our observation that the increased dNTP concentration only partially rescues the sensitivity of the TLS polymerase deletion strain towards the 4-NQO-produced lesions (Figure 2).

Although the TLS polymerases clearly play a major role in the bypass of most DNA lesions, we propose a new pathway, in which the elevated dNTP concentration present in S. cerevisiae after DNA damage engages replicative DNA polymerases (directly or in cooperation with Polζ) in the bypass of certain, perhaps less bulky, DNA lesions. Recently, Lis et al. (34) have proposed a similar pathway that appears to induce mutations at damaged DNA in S. cerevisiae by up-regulating dNTP levels and facilitating translesion synthesis by the replicative Polδ. The DNA-damage-dependent upregulation of RNR transcription in all studied organisms ranging from bacteria to mammals suggests an important role for increased dNTP production during DNA damage. It will be interesting to explore whether replicative polymerases of other organisms are also involved in the bypass of lesions during DNA damage.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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