Carcinogenesis vol.18 no.4 pp.605–610, 1997

COMMENTARY

Which DNA polymerases are used for DNA-repair in eukaryotes?

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There are five well-characterized nuclear DNA polymerases in eukaryotes (DNA polymerases α, β, δ, ε and ζ) and this short review summarizes our current knowledge concerning the participation of each in DNA-repair. The three major DNA excision-repair pathways involve a DNA synthesis step that replaces altered bases or nucleotides removed during repair. Base excision-repair removes many modified bases and abasic sites, and in mammalian cells this mainly involves DNA polymerase β. An alternative means for completion of base excision-repair, involving DNA polymerases δ or ε, may also operate and be even more important in yeast. Nucleotide excision-repair uses DNA polymerases δ or ε to resynthesize the bases removed during repair of pyrimidine dimers and other bulky adducts in DNA. Similarly, mismatch-repair of replication errors appears to involve DNA polymerases δ or ε. DNA polymerase α is required for semi-conservative replication of DNA but not for repair of DNA. A more recently discovered enzyme, DNA polymerase ζ, appears to be involved in the bypass of damage, without excision, and occurs during DNA replication of a damaged template.

Introduction

Knowledge about DNA polymerases in eukaryotes has increased considerably during recent years. In a comprehensive summary in 1985, relevant data regarding DNA-repair polymerases was available only for the mitochondrial DNA polymerase γ and two nuclear DNA polymerases, α and β (1). Currently, there are five characterized nuclear DNA polymerases in eukaryotes, two of them discovered during the last decade (Table I). Which of these nuclear DNA polymerases participate in the different pathways for repair of DNA? This short review summarizes our current knowledge of the subject, with an emphasis on results obtained from mammalian cells and from *Saccharomyces cerevisiae*. The summary answer is that DNA polymerases β, δ and ε, have all been implicated in various forms of DNA-repair, while DNA polymerase ζ is involved in translesion bypass of lesions in DNA. In mammalian cells and budding yeast, DNA polymerase α is required for semi-conservative DNA replication but not for DNA-repair.

The three major DNA excision-repair pathways involve a DNA synthesis step that replaces altered bases or nucleotides removed during repair. These pathways, termed nucleotide excision-repair, base excision-repair, and mismatch-repair are considered in turn below, because each uses a different set of enzymes to carry out the repair process. We also refer to new results on the DNA polymerases involved in translesion bypass of lesions, when DNA replication proceeds through a damaged template without removing the lesion.

Nucleotide excision-repair

Nucleotide excision-repair (NER*) acts with varying efficiencies on a wide variety of adducts in DNA, and is most effective on 'bulky' or helix-distorting lesions. A main function in humans is the removal of UV-induced DNA photoproducts caused by sunlight. Humans affected with the inherited disorder xeroderma pigmentosum (XP) have defects in NER, are hypersensitive to sunlight, and generally show an increased incidence of skin cancers. Seven of the XP gene products form part of the machinery that locates damage in DNA and removes it within an oligonucleotide 24–32 residues long (2,3). A repair patch involving synthesis of ~30 nucleotides (nt) is then formed (Figure 1A).

Nucleotide excision-repair is sensitive to the drug aphidicolin, as shown by experiments with both cells and cell-free extracts (4–8). The known aphidicolin-sensitive DNA polymerases are Pol α (Pol I in yeast), Pol δ (Pol III in yeast), and Pol ε (Pol II in yeast), whereas Pol β is not aphidicolin-sensitive (9). There is good evidence that mammalian DNA polymerase α is not involved in NER. Studies of intact and permeabilized cells have taken advantage of other chemical inhibitors that can differentially inhibit polymerases, and have concluded that DNA polymerase δ or ε, but not α, is responsible for NER synthesis (5–8). In line with this, monoclonal antibodies that neutralize the activity of DNA Pol α in solution, do not significantly inhibit DNA-repair synthesis carried out by cell extracts (4). The main cellular function of DNA polymerase α appears to be an essential role in semi-conservative DNA replication, where the associated primase activity confers the unique ability to synthesize an RNA primer, and the first few nucleotides of each leading strand and Okazaki fragment.

A role for DNA Pol ε or δ is consistent with the requirement for proliferating cell nuclear antigen (PCNA), for nucleotide excision-repair by mammalian cell extracts (10,11). PCNA forms part of a holoenzyme with Pols δ or ε and can stimulate the activities of these enzymes, but not Pol α (12). In yeast and mammalian cells, three identical subunits of PCNA assemble into a toroidal ring (13,14) that encircles the template as a 'sliding clamp' during DNA synthesis. This structure may serve as an anchor at the 3′-OH terminus of a nascent DNA strand, with one face of the ring bound to DNA polymerase. PCNA functions in NER synthesis and in the filling of short single-stranded gaps by assisting in the initiation of DNA synthesis (15,16). PCNA is also known for its ability to confer increased processivity on DNA polymerases, but the latter activity may be unnecessary for filling a 30 nt gap.

There is compelling evidence that PCNA also participates

*__Abbreviations:__ NER, nucleotide excision-repair; BER, base excision-repair; nt, nucleotide; Pol, DNA polymerase; XP, xeroderma pigmentosum; AP site, apurinic, apyrimidinic or abasic site; nt, nucleotide; PCNA, proliferating cell nuclear antigen.

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Table I. Nuclear DNA polymerases

DNA polymerase subunit	Molecular weight (kd)[a]	Human chromosome map position	*S.cerevisiae* gene
Pol α (catalytic)	165	Xp21.3–p22.1	*POL1*
Pol α (middle subunit)	70		*POL10*
Pol α (primase subunit)	58		*PRI1*
Pol α (primase subunit)	49		*PRI2*
Pol β	39	8p11–p12	*POL4*
Pol δ (catalytic)	124	19q13.3–q13.4	*POL3*
Pol δ (small subunit)	51	7	
Pol ε (catalytic)	255 (active fragment of 145)	12q24.3	*POL2*
Pol ε (small subunit)	55		
Pol ζ (catalytic)	173 (*S.cerevisiae*)		*REV3*
Pol ζ (small subunit)	29 (*S.cerevisiae*)		*REV7*

[a]The molecular weight predicted from the open reading frame of the human gene is given, unless otherwise indicated. See Reference (9) for further information and literature citations.

in NER *in vivo*. UV-irradiation of non-S phase human cells in culture increases the level of PCNA that is detectable in nuclei by immunostaining after mild extraction and fixation. This increase occurs rapidly, and is due to a relocalization of PCNA in the nucleoplasm and not to an increase in the total amount of the protein (17–21). A similar increase in non-extractable PCNA is seen in non-proliferating cells of human skin irradiated *in situ* (22). Miura *et al.* found that shortly after UV-irradiation of quiescent normal fibroblast cells, PCNA binds tightly to damaged chromatin in a form that is resistant to detergent and methanol extraction. However, rapid binding of PCNA is absent in UV-irradiated quiescent XP-A and XP-G cells (23–26). This indicates that the association of PCNA with chromatin after irradiation of normal quiescent cells is linked to the NER process. The participation of PCNA in NER suggests that a further DNA polymerase accessory factor, RFC, is also involved. RFC is a multisubunit protein complex that functions during DNA replication to load PCNA onto a DNA template in an ATP-dependent manner (12).

It is not clear whether Pol ε or Pol δ is normally dedicated to nucleotide excision-repair *in vivo*. Perhaps either enzyme can participate in nucleotide excision-repair synthesis in the cell. Indeed, genetic studies of NER in mutants of budding yeast DNA polymerase have recently led to the conclusion that either Pol δ or Pol ε can perform repair of UV-damaged DNA in the absence of the other activity (27).

Using purified enzymes, we studied repair synthesis at gaps created in UV-irradiated DNA molecules by dual incisions during NER (16). Either DNA Pol δ or Pol ε could carry out-repair synthesis, and both polymerases were strictly dependent on both PCNA and RFC under the conditions used (circular DNA substrates, buffer containing 70–80 mM KCl, and the presence of RPA in the reaction mixture). In the presence of DNA ligase I, Pol δ gave rise to a low proportion of complete, ligated products, possibly because of limited strand displacement by the polymerase. The proportion of ligated products with Pol δ can be substantially improved by including DNase IV/FEN1, an enzyme that is discussed in more detail in the next section. Pol ε gave a high proportion of ligated products without DNase IV. A combination of DNA polymerase ε, PCNA, RFC, RPA, and DNA ligase I was used in reactions to reconstitute NER with purified proteins (28), but DNA polymerase δ with PCNA, RFC, DNase IV, RPA and DNA ligase-I will also carry out the reaction (M.K.K.S. and R.D.W., unpublished data).

Nishida *et al.* (6) used an assay involving permeabilized nuclei from UV irradiated cells, and purified a human enzyme, subsequently identified as Pol ε, that was needed for repair synthesis (29). The repair synthesis was dependent on expression of XP genes, showing that it was a consequence of NER. In contrast, Zeng *et al.* (30) found that antibodies against human Pol δ reduced synthesis mediated by nuclear extracts in UV-irradiated DNA, and this type of approach deserves further effort.

Extracts from eggs of the frog, *Xenopus laevis,* can carry out NER in a PCNA-dependent manner, suggesting that Pol δ or ε is also involved in this system (31). However, in oocyte extracts, there is evidence that NER instead requires both Pol α and Pol β (32). It is conceivable that there is a developmental switch in the requirements for DNA polymerases for repair in *Xenopus*.

Base excision-repair

A variety of DNA lesions that require precise and rapid attention are repaired by base excision-repair (BER). These include spontaneous hydrolytic depurination of DNA, deamination of cytosine and 5-methylcytosine, products of reactions with hydroxyl free radicals (generated during normal oxygen metabolism), and formation of covalent DNA adducts by intracellular exposure to small reactive metabolites and coenzymes. Altered bases are first excised in free form by a DNA glycosylase, and the resulting abasic site is corrected by the concerted action of an AP endonuclease, a DNA polymerase and a DNA ligase. Completion of BER requires the removal of the 5′ terminal deoxyribose-phosphate residue generated by the AP endonuclease, accompanied with repair by DNA synthesis and ligation.

The BER process usually involves the replacement of a single damaged nucleotide with a normal residue, but it has emerged that there is a second pathway for completion of BER, involving longer patches of 2–10 residues (Figure 1B). There is good evidence that DNA Pol β is utilized in the short (1 nt) patch mode of repair synthesis during BER in mammalian cells. This has been concluded from studies of uracil-repair by mammalian cell extracts in the presence of various DNA polymerase inhibitors (33); by fractionation of cell extracts to

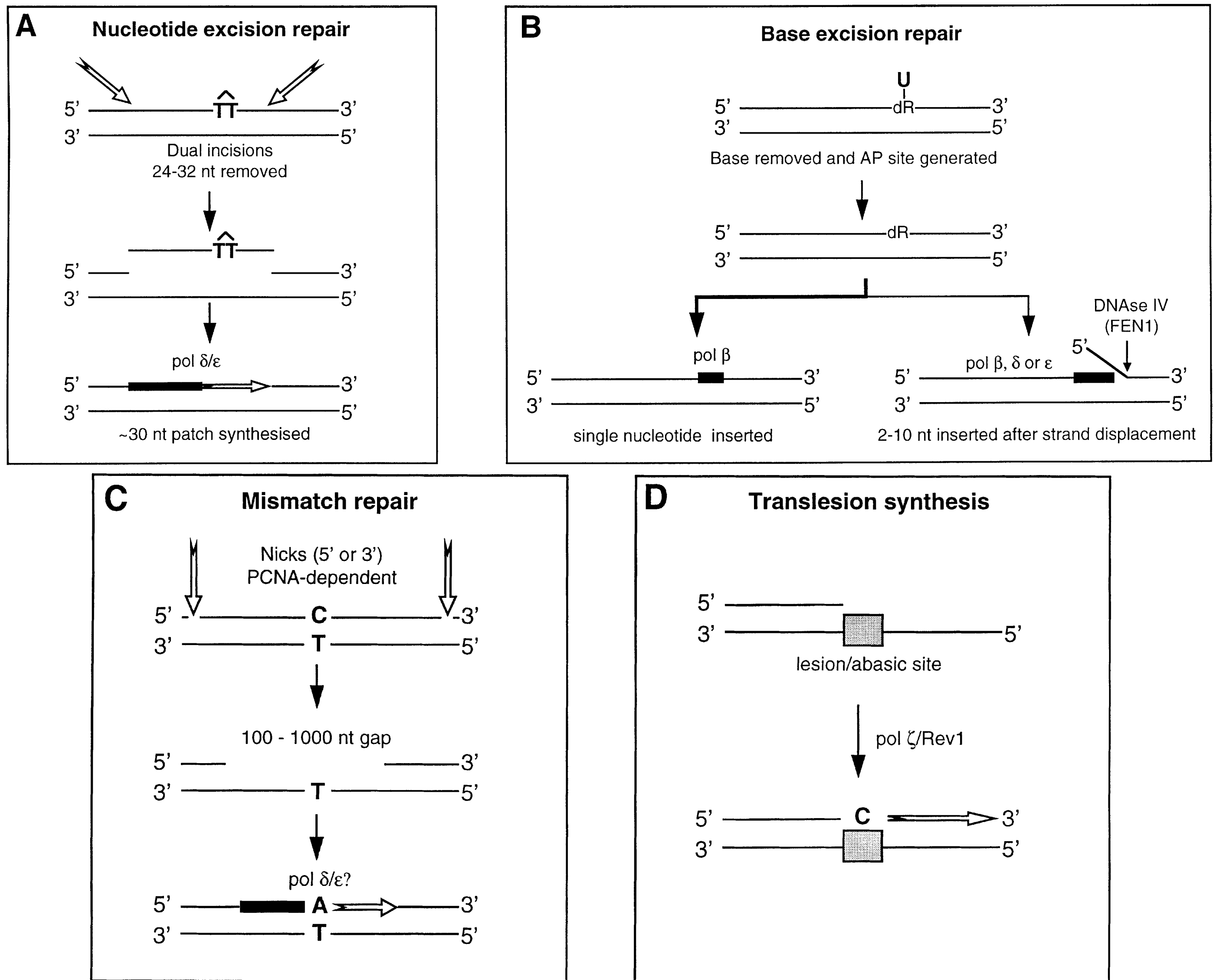

Fig. 1. Schematic summary of the participation of DNA polymerases in (**A**) nucleotide excision-repair, where Pol δ or ε replaces about 30 nt removed after dual incision; (**B**) base excision-repair, a process primarily resulting in 1 nt gap-filling by Pol β but sometimes giving longer patches of 2–10 nt catalysed by Pol β, δ or ε; (**C**) mismatch repair, which can give repair patches of hundreds of nt synthesized by Pol δ or ε; (**D**) translesion bypass synthesis, which is performed most efficiently in *S.cerevisiae* by Pol ζ.

isolate activities that repair natural AP sites or uracil (34–36); by the sensitivity of BER to Pol β antibody (35,37); and by reconstitution of the process with purified enzymes (38). Pol β has two roles in the reaction. The enzyme catalyses the filling in of the one nucleotide gap, but in addition an 8 kd basic domain of the protein has a β-elimination activity that serves to excise the 5′-terminal deoxyribose-phosphate residue at an incised abasic site (39).

Knockout mutation of Pol β in mice causes embryonic lethality (40) and the enzyme is essential for BER in the mice (41). This suggests either that base excision-repair is needed for maintaining viability during embryonic development (by eliminating products of hydrolytic and oxidative degradation), or that Pol β has an additional essential role in development, perhaps in some form of DNA replication (42). In *S.cerevisiae* the situation regarding DNA Pol β is rather different. A 67-kd β-like DNA polymerase has been designated Pol4 (43,44). Yeast strains harbouring a disruption of *POL4* are viable, and exhibit only a weak sensitivity to the simple alkylating agent methyl-methanesulphonate (44). Instead, either DNA Pol δ (45) or Pol ε (46) appears to participate in BER in *S.cerevisiae*. Yeast Pol4 may participate in a form of double-strand break-repair during meiotic recombination (44).

Sometimes, BER is associated with the generation of longer repair patches. These longer repair tracts may result from a nick translation reaction accompanied by strand displacement in the 5→3′ direction, thereby generating a 'flap' type of structure. Such an overhanging 5′ terminal single stranded region of DNA can be removed by the enzyme DNase IV (FEN1). DNase IV was originally found as a 5′→3′ exonuclease that released mononucleotides and short oligonucleotides from DNA (47), and on a flap structure it cleaves a single strand with a 5′ terminus, at the junction with duplex DNA (48). These properties and the sequence similarity

with the 5′→3′ exonuclease domains of eubacterial DNA polymerases (49,50) identify it as a member of the '5′ nuclease' family (51,52). This 43-kd exonuclease is implicated in lagging strand DNA synthesis during semi-conservative replication (53–55). The enzyme has been given several names, including factor pL, MF-1 and FEN1. The name FEN1 is useful as a mnemonic for both '5′ exonuclease' and 'flap endonuclease'.

In contrast to the short-patch pathway, longer patch BER shows a requirement for PCNA, suggesting that the longer patches of 2–10 nt are generated by DNA pols δ or ε (34,56). However, another interpretation is possible, based on the finding that PCNA interacts with and stimulates the activity of DNase IV/FEN1 (57–59), (E. Warbrick, D. Lane, D. Glover and L. Cox, *Oncogene*, in press). Pol β could still carry out longer patch BER, and the role of PCNA might be to stimulate DNase IV/FEN1 activity. Consistent with this, *in vitro* studies have shown that longer patch BER predominates during repair of abasic sites that have been modified by reduction or oxidation (A. Klungland and T. Lindahl, *EMBO J.*, in press). The modified deoxyribose-phosphate residue produced after AP endonuclease cleavage of such sites is refractory to excision by Pol β, but can be removed by DNase IV/FEN1. This reaction is stimulated by PCNA and Pol β then forms repair patches 2–6 nt long.

Repair of mismatches

Mismatch-repair serves to correct replication errors that have escaped the editing process during semi-conservative DNA synthesis, and acts on heteroduplexes formed during recombination between alleles. Homologues of the mismatch-repair proteins MutS and MutL from *Escherichia coli* are used to recognize single base mispairs and unpaired loops of up to four bases (60–62) A mismatched segment is then removed by activities still to be defined, and is replaced by repair synthesis. During mismatch-repair synthesis, the DNA polymerase may need to initiate synthesis of several hundred bases on the 3′ or 5′ side of a mispair. Such bidirectional mismatch-repair by human cell extracts is carried out by an aphidicolin-sensitive DNA polymerase (63). PCNA has also been implicated in mismatch-repair. Yeast PCNA interacts with the primary and secondary mismatch-repair proteins Msh2 and Mlh1 (64) and with the Msh2–Msh3 heterodimer (65). Particular missense mutations in yeast PCNA (64,65) cause a mutator phenotype, which is similar to that seen in *mlh1* mutants, and double mutant analysis suggests that PCNA and Mlh1 are involved in the same mutator pathway. Mismatch-repair activity by human cell extracts was inhibited by the PCNA-binding protein $p21^{Cip1}$ and this inhibition could be overcome by the addition of exogenous PCNA (64). PCNA may have two roles in mismatch-repair. It is likely to serve as an accessory factor for DNA polymerase δ or ε in the resynthesis step (Figure 1C). It is also required at an earlier step in the correction process, perhaps as a signal that helps determine which strand should be repaired (64).

Another possibility for an early role is suggested by the interaction, mentioned in the previous section, between PCNA and DNase IV/FEN1. There are indications that the yeast homologues of DNase IV participate in mutation avoidance. In the fission yeast *S.pombe*, *rad2* mutants are hypersensitive to UV light and show a high rate of spontaneous chromosome loss, indicating a role in lesion-repair, replication, recombination and/or mismatch-repair (52). A related homologue in *S.pombe*, Exo1, also has role in mutation avoidance and mismatch correction (66). Similarly, mutants in a *S.cerevisiae* homologue of DNase IV known as Rad27, Rth1 and Erc11 (67–69) show enhanced instability of certain DNA repeat structures (70), and an increased frequency of duplications (71). It is conceivable that one of the endo- or exo-nucleolytic steps of mismatch repair in eukaryotic cells involves a complex of PCNA with DNase IV/FEN1 or a related enzyme. In *S.cerevisiae*, Rad27 itself is implicated in a pathway of mutation avoidance that is distinct from Msh2-dependent mismatch repair (71). Other homologues of Rad27 exist in the budding yeast genome and it remains to be determined whether they function in mismatch repair.

Replicative bypass of DNA damage

A combination of nucleotide excision repair, base excision repair, and mismatch repair effectively ensures that many mutagenic and cytotoxic lesions are removed. However, replication forks may often encounter damage in the DNA template before it is repaired, and it is clear that many lesions can be bypassed in some way by DNA polymerases. For example, calf thymus Pol δ can bypass up to 30% of UV-induced cyclobutane pyrimidine dimers in a reaction promoted by PCNA (72). In cases where the coding properties of a damaged base are altered or destroyed, such bypass can be mutagenic. Calf thymus Pol β is able to efficiently bypass a d(GpG) cisplatin adduct (73), in a highly mutagenic process that most frequently creates single base deletion mutants (74). Human Pol β can also bypass abasic sites in DNA, resulting in both deletion and base substitution misincorporation errors (75).

In *S.cerevisiae*, at least three different proteins (Rev1, Rev3 and Rev7) are implicated in a pathway of DNA damage-induced mutagenesis that involves bypass of lesions in DNA (76). The *REV3* gene encodes a protein related at the primary sequence level to the other nuclear DNA polymerases, and is tightly bound to the Rev7 protein (76). The Rev3-Rev7 complex is designated yeast DNA polymerase zeta (Pol ζ). Pol ζ can bypass a thymine–thymine cyclobutane dimer and has a much higher capability for translesion synthesis than does Pol α (76). Another gene product involved in this translesion synthesis pathway, Rev1, has deoxycytidyl transferase activity which transfers a dCMP residue from dCTP to the 3′ end of a DNA primer in a template-dependent reaction (77). An insertion of a C residue opposite an abasic site can be efficiently extended by Pol ζ but not Pol α. The Rev1 transferase activity may therefore prepare the DNA for translesion bypass by Pol ζ by inserting dCMP opposite an abasic site and facilitate the ability of Pol ζ to traverse a lesion (Figure 1D). Interestingly, mutations in *REV3* not only suppress damage-induced mutagenesis, but also decrease spontaneous mutation in yeast to about one-fifth of normal (78). This implies that most spontaneous mutations in *S.cerevisiae* are associated with mutagenic bypass of DNA lesons that escape DNA repair, rather than with misincorporation opposite normal bases by replicative DNA polymerases during semi-conservative DNA synthesis. Translesion DNA synthesis also occurs in mammalian cells and cell extracts (79–83), and it will be interesting to see if the mammalian counterpart of Pol ζ plays a role in this process.

Figure 1 summarizes the participation of the various DNA polymerases in DNA repair and translesion bypass.

Acknowledgements

We thank John Diffley, Elizabeth Evans and Tomas Lindahl for comments, and Tony Coe and Tony Hymas for assistance in preparing the manuscript.

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Received on January 10, 1997; accepted on January 21, 1997