Base excision repair fidelity in normal and cancer cells

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In mammalian cells, base excision repair (BER) is the major repair pathway involved in the removal of non-bulky damaged nucleotides. The fidelity of BER is dependent on the polymerization step, where the major BER DNA polymerase (Pol β) must incorporate the correct Watson–Crick base paired nucleotide into the one nucleotide repair gap. Recent studies have indicated that expression of some Pol β variants or changes in expression of wild-type Pol β protein, frequently found in cancer cells, can lead to DNA repair synthesis errors and confers to cells a mutator phenotype.

Introduction

Genome integrity can be threatened by environmental agents such as ultraviolet (UV) light, ionizing radiation, carcinogens and cytotoxic compounds, as well as by endogenous factors, such as reactive oxygen species (1,2). Consequently, DNA is protected by various DNA repair mechanisms such as base excision repair (BER) and nucleotide excision repair to prevent mutations. The fine balance between genome stability and instability must be tightly regulated since mutations are central to the progression of disease, ageing and evolution.

In mammalian cells, the highly conserved BER pathway is the major cellular defence against non-bulky damaged nucleotides, abasic sites and single-strand breaks generated by reactive oxygen species, alkylating agents and ionizing radiation (2). BER is a multiprotein pathway initiated by damage-specific DNA glycosylases which recognize and cleave the N-glycosyl bond connecting the aberrant base to the sugar–phosphate backbone, creating an abasic or apurinic/apyrimidinic (AP) site (3). Presently, 11 DNA glycosylases have been implicated in mammalian BER and they effectively cover repair of the majority of DNA base lesions. AP sites generated by spontaneous hydrolysis of the N-glycosyl bond (~10 000 per mammalian cell/day) (4) or as a result of DNA glycosylase action are highly mutagenic and cytotoxic as they are non-coding (5–7). Next, AP endonuclease 1 (APE1) recognizes AP site and cleaves the DNA phosphodiester backbone on the 5′ side of the AP site, leaving a 3′-hydroxyl group and a 5′-deoxyribose phosphate (dRp) group flanking the nucleotide (nt) gap (8,9). Further repair can proceed via two subpathways that utilize different subsets of enzymes; (i) short-patch BER involves replacement of 1 nt or (ii) long-patch BER that involves gap-filling of 2–6 nt (10). Both subpathways are initiated by DNA polymerase β (Pol β), the major BER DNA polymerase, which adds the first nucleotide into the repair gap. During short-patch BER (Figure 1) Pol β adds 1 nt into the repair gap and simultaneously removes the 5′-dRp moiety by its intrinsic dRp lyase activity (11). Finally, the nick containing 3′-hydroxyl and 5′-phosphate ends are sealed by DNA ligase IIIα–XRCC1 heterodimer. However, modification of the 5′-dRp moiety by oxidation or reduction prevents Pol β from excising it and the long-patch BER mechanism, or ‘proliferating cell nuclear antigen (PCNA)-dependent BER’ is employed (12). At this point, Pol β is replaced by Pol δ/ε, which extends the repair patch and displaces several nucleotides to create a 5′-flap junction (13). Flap endonuclease-1 (FEN-1) cleaves the flap at the single-strand/double-strand DNA junction and the DNA ends are then sealed by DNA ligase I. All these reactions have been found to be stimulated by PCNA (10). Among the different enzymatic activities participating in both short- and long-patch BER, the DNA synthesis step is the most likely source of generating mutations during BER. Therefore, the ability of DNA polymerases involved in BER to perform error-free repair synthesis has been addressed in many studies. This review will focus primarily on the fidelity of mammalian BER.

DNA polymerases involved in BER

Faithful replication by DNA polymerases is essential in maintaining genome integrity during DNA repair and recombination. Each DNA polymerase is presented with the same task of selecting the sole correct Watson–Crick base paired substrate from a pool of four structurally similar deoxynucleotide triphosphates (dNTPs), and successfully incorporating it onto the growing DNA strand.

Currently, over 19 different eukaryotic DNA polymerases have been identified (14). Among those, Pol α, δ and ε are the major mammalian replicative DNA polymerases. These DNA polymerases have relatively high fidelity. In situations where the replication machinery encounters a DNA lesion, translesion DNA polymerases (Pol ζ, η, ι, κ and Rev1) assist replication bypass of the lesion to avoid collapse of the replication fork (reviewed in ref. 15). Depending on the DNA polymerases involved, translesion synthesis may be quite accurate or error-prone. For example, Pol ζ and η are involved in two independent pathways: error-prone and error-free bypass of thymine–thymine cyclobutane (TT) dimers, respectively (16,17).

Three or four DNA polymerases are thought to be involved in different BER pathways; Pol β, δ, ε and possibly Pol λ, where Pol β is the major BER polymerase (18–20). Pol β is a 39 kDa monomeric protein comprising two catalytic domains connected by a protease-sensitive hinge region; the N-terminal 8 kDa dRp lyase and DNA binding domain, and the C-terminal...
31 kDa DNA polymerase domain (reviewed in ref. 21). The protein also consists of several subdomains necessary for catalysis; the fingers, the thumb and the palm (11,22). In contrast to replicative DNA polymerases, Pol β lacks any intrinsic 3'-5' exonuclease activity and is incapable of proofreading. As previously mentioned, Pol β is the major DNA polymerase involved in short-patch BER (20) but Pol β also initiates long-patch BER by adding the first nucleotide into the repair gap (12). However, further DNA synthesis during long-patch BER is conducted by Pol δ/ε which further incorporate 2–10 more nucleotides (10,18,23).

Mammalian Pol δ and ε have similar roles in DNA replication and repair; however, their biochemical properties differ. Pol δ requires two other factors; PCNA and replication factor C (RF-C) for efficient DNA synthesis (24). In contrast, Pol ε is highly processive in the absence of PCNA (25). Pol δ is comprised of four subunits (125, 66, 50 and 12 kDa) (26) and Pol ε is a heterodimer with a 215 kDa catalytic subunit and 55 kDa subunit with unknown function (27). Both Pol δ and ε contain 3'-5' exonuclease proofreading activity and are able to remove misincorporated nucleotides (28).

The newest member of the X family of DNA polymerases, Pol λ may also play a backup role in BER (29). Mammalian Pol λ shares 32% amino acid homology with Pol β (30) and has an intrinsic dRp lyase activity (31). Pol λ was suggested to be responsible for background repair activity in Pol β-deficient cell extracts (19), implicating a possible role in BER. However, the major role of Pol λ is still undefined since Pol λ is also involved in processing of DNA ends during non-homologous end joining (32–34).

**DNA polymerase synthesis fidelity assays**

Three major approaches have been used to address DNA synthesis fidelity of purified DNA polymerases: (i) direct measurement of nucleotide misincorporation frequency using fluorescent or radioactively labelled nucleotide triphosphates; (ii) primer extension reactions using oligonucleotide duplex substrates with a 5'-labelled template; or (iii) reporter gene-based systems where misincorporation into bacteriophage or plasmid DNA during *in vitro* DNA synthesis is tested as reverse or forward mutations of a reporter gene, e.g. *lacZ* after transformation of template DNA into bacterial cells.

Initially, synthetic homopolymer polynucleotides were used as DNA polymerase synthesis templates (35). Although the assay was fairly high-throughput, the repeating nature of the polynucleotide promotes slippage of the primer along the template during synthesis. To overcome this problem, assays using synthetic oligonucleotide templates were developed and proved to be efficient substrates for DNA synthesis studies (reviewed in ref. 36).

Biological assays with natural DNA templates were based on analysis of reversions or direct mutations arising during DNA synthesis. Defined single base substitution mutations were introduced into the amber locus (am3) of φX174 bacteriophage DNA and errors during DNA synthesis were detected after transfection of the template DNA in bacterial cells by determining the reversion frequency of the mutation to wild type (37). This assay was 2–3 times more sensitive than the polynucleotide assays and provided more realistic misincorporation rates; however, analyses were restricted to measuring base substitution errors only.
Spontaneous and induced mutation rates reflect several different types of errors and often occur at different sites within a gene, so in order to monitor a wide spectrum of errors, the assay was adapted and simplified by Kunkel's group (38) who developed an M13mp2/lacZa DNA-based assay to measure DNA synthesis fidelity in vitro. This assay monitors DNA synthesis of a target sequence located within the lacZα gene and is performed using purified DNA polymerase and a gapped DNA substrate. The size of the gap may vary from 5 to 390 nt (38,39). After repair synthesis, substrate DNA is transfected into bacterial cells and any errors causing loss of lacZα gene function can be observed as colourless plaques on indicator plates. This forward mutational assay is capable of detecting frameshift, deletion, duplication and complex errors in addition to a large number of different base substitution errors at many sites and consequently has proved to be a powerful and widely used tool.

More recently, this in vitro forward mutation assay has been further developed by Eckert and co-workers to measure DNA damages on the herpes simplex virus type-1 thymidine kinase (HSV-tk) gene, using chloramphenicol selection (40). This system allows mutagenesis to be monitored in both bacterial and human cells (40).

**Fidelity of repair synthesis by purified/recombinant BER DNA polymerases**

As previously mentioned, Pol β is involved in both BER pathways by inserting the first nucleotide into a repair gap. In early studies Pol β fidelity was measured using substrates with long (up to 300 nt) gaps (38) or in primer extension reactions using duplex oligonucleotide substrates (41). Both the efficiency and fidelity of Pol β DNA synthesis was found to be reduced (average mutation frequency, $10^{-6}$) compared to replicative DNA polymerases (average mutation frequency, $10^{-8}$) (42). So, for many years Pol β was considered as a low fidelity DNA polymerase (36). However, since Pol β plays the major role in short-patch BER one can assume that Pol β has evolved as a distributive polymerase designed to fill 1 nt gaps containing 3'-hydroxyl and 5'-phosphate ends. Indeed, Pol β requires a phosphate group at the 5'-margin of the gap and several studies have shown that 5'-phosphorylated 1 nt gapped DNA is the preferred substrate for Pol β (41,43,44). Nucleotide misincorporation by Pol β is 10–100-fold lower on 5'-phosphorylated 1 nt gapped DNA compared with recessed, 6 nt gapped, 5'-phosphorylated 6 nt gapped and unphosphorylated 1 nt gapped substrates (44). As Pol β has no proofreading activity, its incorporation accuracy results exclusively from the nucleotide selectivity of the enzyme. Unlike most replicative polymerases, which select the correct dNTP through an ‘induced fit’ mechanism, Pol β fidelity is determined through specific interactions between the enzyme and the template base at the active site. In the induced-fit mechanism, binding of the correct incoming nucleotide and two metal ions within the active site creates a substrate-induced conformational change from an open state to a closed state, which then activates the polymerase for catalysis and hence DNA extension (45). However, nucleotide discrimination by Pol β appears to be determined by steric exclusion against the incorrect substrate, the precise positioning of the DNA duplex terminus, templating and incoming nucleotides and the catalytic residues within the active site of Pol β (46). Imprecise positioning of active site residues or DNA can result in misincorporation of nucleotides into DNA. Amino acid residues both distant and near to the Pol β active site influence its geometry, suggesting that the movements and positioning of subdomains of Pol β have a significant impact upon its fidelity and consequently its catalytic efficiency (46).

Presently, it is not clear which DNA polymerase, Pol δ or ε, is contributing more to long-patch BER, since both enzymes can catalyse strand-displacement synthesis which is required for this pathway (18,47,48). However, both Pol δ and ε have shown very high fidelity DNA synthesis in the in vitro systems (42).

**Mutation spectrum of BER-induced mutations**

The M13mp2/lacZα forward mutation assay has been widely used to investigate BER fidelity of human cell extracts for a variety of different lesions such as uracil (49), single oxygen-induced and gamma radiation-induced lesions (50,51) and gapped DNA where the gap size may vary from 1 to 390 nt (38,39). In the majority of the assays used, the DNA substrate contains site-specific lesion(s) positioned within a coding region of the lacZα gene and after repair in cell extracts any arising changes in the DNA sequence are detected as a loss of gene function, hence loss of β-galactosidase expression. Repaired bacteriophage DNA substrates are purified and transformed into bacterial cells, mutant phages (colourless) can be isolated and sequenced to identify mutations.

Several independent studies have found that although different types of mutations can arise during short-patch BER, the majority of mutations were single nucleotide deletions (41,52–54).

**Models for replication errors involving strand misalignments**

Two major models have been utilized to explain the occurrence of 1 nt frameshifts during DNA replication: (i) the majority of sequence-dependent models are based on the ‘template-primer slippage’ model, proposed by Streisinger in 1985 (55), that does not include an active role of DNA polymerase in mutation formation but involves strand displacement during replication of short repeat sequences. Polymerization proceeds through the repetitive sequence and slippage of the template and primer strands produces misaligned intermediates that can be stabilized by correct base pairs, (Figure 2A). (ii) The second group of models include an active role of DNA polymerase during mutation formation. For example, the ‘dNTP-stabilized misalignment’ model has been proposed where the templating base is bypassed by the polymerase and a nucleotide complementary to an adjacent downstream template site is incorporated (56), (Figure 2B).

Interestingly, DNA sequence context has a profound effect on the resulting mutation spectrum during repair of long gaps (up to 300 nt) and multiple mechanisms of frameshift formation are likely to be involved (38). However, ‘dNTP-stabilized misalignment’ may be the predominant mechanism during BER since 1 nt frameshifts (the major class of mutations for both BER pathways) were induced independently of sequence context (41,52–54).

**DNA polymerase imbalance: effects on replication fidelity and genome stability**

We proposed earlier that dNTP-stabilized misalignment is most probably involved in frameshift formation during BER (54). According to this mechanism, incorporation of the new nucleotide to an adjacent template site downstream of the repair gap generates 1 nt frameshifts (Figure 3). As a distributive
Increasing the probability of frameshift formation. Indeed, enzyme (even operating as a distributive enzyme) may add more than one nucleotide into the misaligned phosphodiester bond. A misaligned intermediate that can be stabilized by correct base pairs. (This model of enzyme is present, then Pol β has been proposed where the templating base is bypassed by the polymerase and a nucleotide complementary to an adjacent downstream template site is added.) The second group of models include an active role of DNA polymerase during mutation formation. For example, the 'dNTP-stabilized misalignment' model has been proposed where the templating base is bypassed by the polymerase and a nucleotide complementary to an adjacent downstream template site is incorporated.

**Fig. 2.** Models for 1 nt frameshifts (adapted from ref. 38). Two major models have been utilized to explain the occurrence of 1 nt frameshifts during DNA replication: (A) The majority of sequence-dependent models are based on the 'template-primer slippage' model that does not include an active role of DNA polymerase in mutation formation but involves strand displacement during replication of short repeat sequences. Polymerization proceeds through the repetitive sequence and slippage of the template and primer strands produces misaligned intermediates that can be stabilized by correct base pairs. (B) The second group of models include an active role of DNA polymerase during mutation formation. For example, the 'dNTP-stabilized misalignment' model has been proposed where the templating base is bypassed by the polymerase and a nucleotide complementary to an adjacent downstream template site is incorporated.

**Fig. 3.** Model depicting formation of 1 nt deletions during BER. A DNA glycosylase removes the damaged base (shown as X), then APE1 cleaves the phosphodiester bond 5’ to the AP site and generates a single-strand break with 3’-hydroxyl and 5’-sugar phosphate ends (dRp). Slippage of the DNA polymerase during addition of the first nucleotide (T shown in bold) into the repair gap generates 1 nt deletions. Addition of the second nucleotide (A shown in bold) stabilizes the deletion loop and generates a three-residue flap that includes the dRp residue and the two displaced nucleotides. After removal of the flap by FEN1 and subsequent ligation, a DNA replication cycle will therefore result in a 1 nt deletion.

DNA polymerase β variants: effects on replication fidelity and genome stability

Pol β variants affecting control of incoming nucleotide selection may be a major source of mutations during BER. Direct sequencing of the pol β gene from different cancer cells identified a number of mutations. To date, 189 various tumours have been characterized and 54 (30%) of these express variants of the Pol β protein (60,66), pol β gene mutations have been identified in human prostate, gastric, colorectal, lung, breast and bladder cancers (60,67–73). Single amino acid alterations (48%) were the most prevalent mutations but alternative splice variants of exon 11 (26%), multiple variant forms (14%) and truncated forms (12%) of Pol β were also observed (66).

To investigate whether these Pol β mutations are increasing the spontaneous level of mutations, Sweasy and co-workers (74) have extensively studied the fidelity of DNA synthesis and transformation activity of colon (K298M) and prostate (I260M) cancer-associated Pol β variants. The Ile260 residue is located in the hydrophobic hinge region of Pol β, which is important for the closing of the polymerase upon interaction with its nucleotide substrate (22). Therefore, amino acid alterations at key Pol β residues impaired DNA synthesis and resulted in a mutator phenotype. They found that expression of the tumour variants in mouse cells resulted in cellular transformation, which was maintained even after abolition of Pol β variant expression. In contrast, expression of wild-type Pol β did not induce a transformed phenotype in mouse cells, thus suggesting that Pol β variants may induce mutations into cells
during the gap-filling step of BER (75). Banerjee and co-workers (68,70) have been investigating an alternative splice variant of Pol β where 87 nt are deleted (exon 11) in the catalytic domain. Expression of the truncated (36kDa) Pol β protein has been demonstrated in colorectal and breast cancers, suggesting that dominant negative mutants of Pol β may interfere with normal repair and as a result induce genetic instability and sensitivity to DNA damaging agents. Indeed, transformation of cells with dominant negative mutants of Pol β resulted in radiosensitivity (76).

Clearly, alterations to Pol β whether it is changes in expression or gene mutation have an adverse effect on cells and can induce mutations, possibly by altering the efficiency and/or fidelity of gap-filling during BER.

References


