

## Chapter 2

# Base Excision DNA Repair: The House Keeping Guardian for Genomic Stability in the Brain

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**Abstract** During evolution, organisms have developed a number of strategies to keep the structure of their genetic material intact. However, evolutionary forces keep driving metabolic changes that would lead to more viable progenies for survival and propagation. Thus natural evolution uses two opposing forces to improve the genetic makeup of the species. With the advent of organisms turning aerobic, the advantage of gains in energy production also paved the way to cope up with increasing oxidative damage from within. In response, the system has developed ways and means to offset the possibilities of oxidative damage to macromolecules and in particular, the genetic material, DNA. Of the many pathways of DNA repair, the base excision repair (BER) stands out as the watch dog to alert even a minor modification to a base in DNA and also to initiate the process of repair. Thus, BER plays a seminal role in maintaining the structural integrity of DNA that is quite often threatened from events taking place within the cell itself. Implicit in this crucial role is also the fact that any perturbation in this repair pathway could lead to deleterious consequences—particularly in a post-mitotic tissue like brain. It is becoming increasingly apparent that BER has close correlation with the age-dependent debilities/disorders.

**Keywords** Base excision repair • Brain • DNA damage • Aging • DNA polymerase  $\beta$

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## 2.1 Introduction

The basic prerequisites for the survival and proper functioning of the living organisms are the accurate maintenance and transmission of the genetic instructions coded in DNA, with the highest possible fidelity. The faithful course of these processes is guaranteed by the genomic stability, which ensures both cellular homeostasis and genetic continuity in multicellular organisms. The integrity of the genetic material is under constant attack by numerous exogenous and endogenous sources. Consequently, formation of DNA damages is a common event in living cells. Accordingly, it is not surprising that during the evolution, an intricate network of sophisticated, interwoven DNA repair systems has developed. The various features of DNA damages require different ways to counteract their deleterious effects. However, mutagenesis is a prerequisite for evolution and some adaptive genomic alterations escape the watchful security of DNA repair system. Sometimes these mutations can lead to increasing vulnerability for diseases like cancer and phenomenon like aging. It thus appears that DNA repair potential in organisms plays a very crucial and fundamental role in conserving the genomic integrity in any given species as well as allowing the evolution as and when necessary.

With the original findings of Alexander (1967) that DNA repair is at a low key once the cells are differentiated into the post-mitotic state, the manner in which the genomic maintenance is achieved in a post-mitotic but metabolically very active organ like brain has become a curious issue. Subsequent studies have indicated, however, that even in adult brain a certain level of DNA repair exists, and it was realized that the terminally differentiated nature of brain cells offers a good model for studying the repair pathways and the accumulation of genomic damage during the lifespan of a given animal.

The purpose of this article is to review an outline of the various types of structural alterations that are known to occur in genomic DNA of mammalian cells and discuss how the base excision repair (BER) pathway operates to counteract such DNA damage, with a special emphasis on the brain tissue.

## 2.2 The Vulnerable Nervous System

The nervous system consists of two classes of cells, the neurons and glial cells, which include astrocytes, oligodendrocytes and microglial cells. Further, in almost all the species, central nervous system is one of the earliest systems to develop and differentiate. It would therefore follow that a neuronal cell in brain of any species, at any given point of time, is almost as old as the animal itself. An important feature of neuronal cells is their post-mitotic state, i.e., unlike other cell populations (e.g. skin, blood, connective tissue, and even glial cells); neurons are terminally differentiated (i.e. non-dividing), and therefore, devoid of chromosome replication. However, it is also known that neurons are one of the most metabolically active cells in the body.

Hence, they need elaborate, stringent defense mechanisms to ensure their longevity. Neurons display high rates of transcription and translation, which are associated with high rates of metabolism and mitochondrial activity. The amount of oxygen consumed by the brain, relative to its size, exceeds by 20 % that of other organs (Clark and Sokoloff 1999). This high activity, coupled with high oxygen consumption, creates a stressful environment for neurons that includes noxious metabolic by-products, primarily reactive oxygen species (ROS), constantly attacking neuronal genomic and mitochondrial DNA (Barzilai 2007, 2010; Fishel et al. 2007).

## 2.3 DNA Damage in Brain

DNA is vulnerable to attack by other chemicals, which can result in alterations in its coding properties or normal function in transcription or replication (Subba Rao and Loeb 1992; Lindahl 1993). The native structure of DNA is subject to damage by both endogenous and exogenous events. DNA damage can result in loss or modification of bases, single-strand breaks (SSBs) and double strand breaks (DSBs), the production of mismatched base pairs, DNA-DNA crosslinks and crosslinks between DNA and other cellular constituents (Rao 1993; Barnes and Lindahl 2004). Many of these DNA modifications have been shown to be mutagenic in vitro and in vivo (Loeb 1989). In view of the generally protected situation of brain (including the blood-brain barrier), the main enemy for causing DNA damage in brain cells is from within only. The number of ways the nuclear DNA in brain cells could possibly be damaged is in detail described by Rao (1993).

Endogenous sources of DNA damage and mutations can be classified into three groups. First, DNA is subjected to spontaneous hydrolytic processes, including depurination, depyrimidination, deamination and cleavage of phosphodiester bonds. Of these, depurination is the most frequent process. From the rate of depurination of DNA in aqueous solution under physiological conditions in vitro, it has been estimated that the DNA in each cell undergoes 10,000 depurinations per day (Lindahl and Nyberg 1972). The hydrolytic damage to DNA is the deamination of DNA bases, in particular at cytosine yields uracil, which is recognized by a specific repair enzyme uracil DNA-glycosylase (UDG) and uracil in DNA is known to be removed with very high efficiency. In contrast, deamination of 5-methyl cytosine yields thymine, a normal base in DNA. Possibly, because of this reason, G-T mispairs are repaired rather less efficiently than G-U mispairs and as a consequence, 5-methyl cytosine residues are hot spots for mutation in cells (Duncan and Miller 1980; Ehrlich et al. 1990).

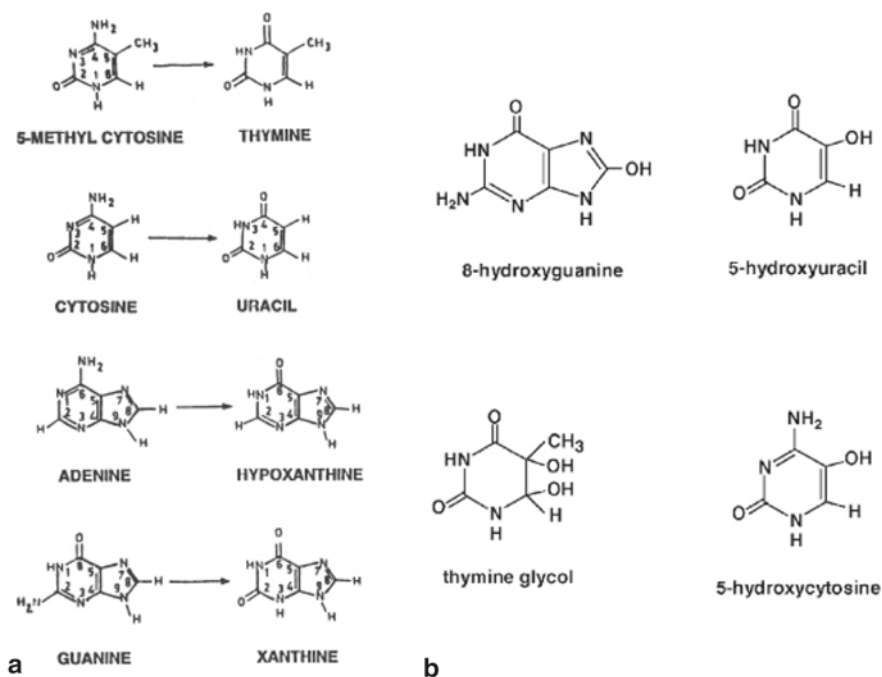
A second major source of damage to cellular DNA is the formation of reactive derivatives of oxygen from normal metabolic process and by exposure of cells to exogenous agents (Demple and Harrison 1994; Barnes and Lindahl 2004). Such ROS includes reactive methyl and ethyl groups, lipid peroxides and oxygen free radicals. It is becoming increasingly apparent that oxidative reactions may be a major source of DNA damage. Oxygen is metabolized by a series of single electron

**Table 2.1** Approximate frequencies of occurrence of DNA damages in mammalian cells

Type of damage	Events per day/cell	Reference
Depurination	10,000	Lindahl and Nyberg (1972)
Deprimidination	500	Lindahl and Karlstorm (1973)
Deamination	100–300	Lindahl and Nyberg (1974)
Base damages (including all types of base damage viz. oxidative damage, adduct formation with reducing sugars, methylation, crosslinks, and so forth)	10,000	Richter et al. (1988)
Single-strand breaks	20,000–40,000	
Double strand breaks	9	Bernstein and Bernstein (1991)
Interstrands crosslinks	8	Bernstein and Bernstein (1991)
DNA protein crosslinks	Unknown	Bernstein and Bernstein (1991)

reductions, yielding highly reactive species including hydroxyl radicals, hydrogen peroxide, superoxide and singlet oxygen. Ionizing radiation can also generate reactive radicals both by direct attack on DNA and by the radiolysis of H<sub>2</sub>O (Halliwell 1992; Riley 1994; Bandyopadhyay et al. 1999). The range of DNA lesions generated by ROS is extensive and more than 20 products of base oxidation have been identified (Cooke et al. 2003). Modified purine and pyrimidine bases constitute one of the major classes of hydroxyl radical mediated DNA damage together with oligonucleotide strand breaks, DNA-protein crosslinks and abasic (AP) sites (Cadet et al. 1999). The products of such reactions, e.g., 8-oxoguanine (8-oxoG), formamidopyrimidines and ring-saturated pyrimidines such as thymine glycol, are often mutagenic or cytotoxic (Wilson et al. 2003; Evans et al. 2004). Considering that ROS produces multiple modifications in DNA, it can be estimated that as many as 10<sup>4</sup>–10<sup>5</sup> bases in DNA in each human cell are replaced as a result of oxygen free radical damage every day. Third, though neurons are non-replicative cells, DNA synthesis occurs during the repair of DNA damage. This repair synthesis is believed to be catalyzed in part by DNA polymerase  $\beta$  (pol  $\beta$ ), the most error prone of the mammalian DNA polymerases (Loeb and Kunkel 1982). Considering that brain is an organ with unusually high oxygen consumption, these aspects of genetic damage can be expected to play a greater role in brain than in other tissues.

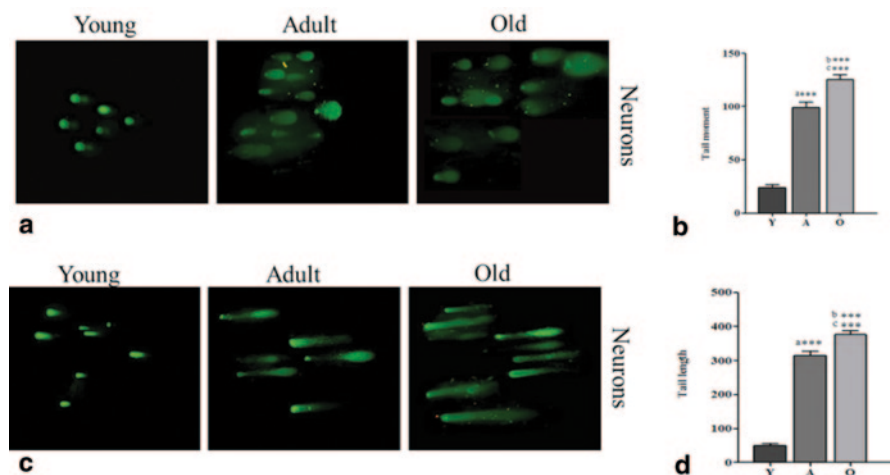
The frequency with which the DNA damaging events occur in a mammalian cell has been examined by a number of workers, and this information is summarized in Table 2.1, and some of the DNA base alterations are as shown in Fig. 2.1. Work from our laboratory, using two biochemical approaches, has demonstrated that neurons from different regions of rat brain harbor both SSBs and DSBs in their nuclear DNA, and this strand breaks increase with age of the animal (Mandavilli and Rao 1996a, b). However, we have recently accessed genomic DNA damage of brain cells on a single-cell basis, popularly called “comet assay”. By adjusting the pH as well as experimental conditions, the types of DNA lesions that are detectable by comet assay include AP sites (alkali-labile sites), SSBs, DSBs and DNA-protein



**Fig. 2.1** Different forms of DNA damage that could occur due to various endogenous or/and exogenous factors. **a** Some of the deamination product of the DNA bases. **b** Some of the oxidized products of DNA bases

crosslinks (Tice et al. 2000). The alkaline denaturation of DNA and electrophoresis at pH > 13 is generally considered capable of detecting DNA SSBs, AP sites, DNA-DNA/DNA-protein crosslinking, and SSBs associated with incomplete excision repair sites (Singh et al. 1988; Tice et al. 2000). Neutral conditions (pH ~ 8.3) are used to detect mostly DSBs because DNA remains double stranded under this condition, and regions containing DSBs migrate more readily in an electrophoretic field (Ostling and Johanson 1984; Singh et al. 1988; Lemay and Wood 1999).

As it can be seen in Fig. 2.2a, the alkaline version of the comet assay is used to determine the level of DNA strand breaks in isolated neurons prepared from animals of different age groups. After SYBR Green I staining, young neuronal nuclei appeared very bright and round (Fig. 2.2a). Furthermore, very little migration of DNA is evident; suggesting that DNA damage is at its minimum in young neurons. However, with advancement of age (adult and old), there is significant relaxation and migration of DNA from the nucleus, forming a “comet” tail (Fig. 2.2a). It is also clear that the tail moment values increased with age. The tail moment value, in comparison with that in young neurons increased 4.1-fold in adult, and 5.1-fold in old neurons (Fig. 2.2b). Closer evaluation of the data reveals that a considerable



**Fig. 2.2** Comet assay of neurons prepared from ‘young’ (7 days postnatal), ‘adult’ (6 months) and ‘old’ ( $\geq 24$  months) rat brain. **a** Fluorescence photomicrographs showing comets of neurons (alkaline condition). **b** Bar graph showing tail moment expressed in arbitrary units in neurons prepared from young, adult and old rat brain cortex. Values at **a** and **b** are significantly different from the corresponding values at young. **c** is significantly different from the corresponding values at adult. **c** Fluorescence photomicrographs showing comets of neurons (neutral condition). **d** Bar graph showing tail length expressed in arbitrary units in neurons. Values at **a** and **b** are significantly different from the corresponding values at young; **c** is significantly different from the corresponding values at adult. \*\*\* $p < 0.001$  (Adapted from Swain and Subba Rao 2011)

number of strands break accumulated by adulthood itself (6 months) with the further increase in the strand breaks in old age in both types of cells.

We have also examined neutral version of the comet assay without treatment with alkaline buffer for electrophoresis. Under these conditions, the comet is considered to detect largely, if not exclusively DSBs. The extent of DNA damage measured as tail length under these conditions also increased in neurons with age (Fig. 2.2c). The tail length value of adult neurons increased 6.2-fold in comparison to that in young while in the case of old neurons, there was a 7.4-fold increase in comparison to young neurons (Fig. 2.2c and d).

The above comet assay method measures different DNA strand breaks by changing the pH condition. Strand breaks cause relaxation of the supercoiling in the DNA, and free DNA loops are pulled towards the anode during electrophoresis giving the appearance of a comet tail. Fluorescence staining enables DNA damage to be visualized and quantified. However, in this method damage or alteration of DNA base could not be quantified. To make the assay more sensitive for determination of damaged base in nuclear DNA specifically, we have modified the alkaline version of the comet assay by incorporating a lesion-specific enzyme which increases its activity through the recognition of the damaged base substrate and thus introducing additional breaks causing enhanced DNA relaxation and migration (Swain and

**Table 2.2** Measurement of OGG1 sensitive sites in young (7 days postnatal), adult (6 months) and old ( $\geq 2$  years) rat brain neurons by FLARE™ comet assay. (Adapted from Swain and Subba Rao 2011)

Sample	Tail moment		Net amount of OGG1 sensitive series
	Mean±SEM		
	Buffer	hOGG1	
Young neurons	14.46±1.42	22.75±2.96	8.29
Adult neurons	94.76±4.68	138.18±11.04 <sup>a***</sup>	43.42
Old neurons	114.01±3.00	175.64±7.77 <sup>b,c***</sup>	61.63

a and b are significantly different from the corresponding values at young. c is significantly different from the corresponding values at adult

\*\*\* $p < 0.001$

Subba Rao 2011). When using lesion-specific enzymes to measure DNA damage, the usual practice is to incubate a slide (two gels) with buffer alone in parallel along with the +enzyme slide, and to subtract the mean comet score of the control (buffer) slide from the mean score of the +enzyme slide (Collins 2009).

We have recently measured the presence of 8-oxoguanine DNA-glycosylase 1 (OGG1) sensitive sites in DNA of neurons by introducing human OGG1 in the assay on naked DNA after lysis was performed.

Human OGG1 initiates the repair of 8-oxoG bases by excising them and cutting the sugar-phosphate backbone of the DNA molecule. Thus additional strand breaks are induced at the location of oxidized base substrate, causing additional DNA relaxation and migration. The outcomes from these studies are shown in Table 2.2. When neurons were incubated with only buffer, an increase of DNA migration in the tail was observed with increasing age of the animal (Table 2.2). This increase in tail movement is taken to indicate increasing DNA damage that occurs due to aging. When the cells were incubated with buffer containing human OGG1, there was a further increase in the tail movement, which must be due to the removal of 8-oxoG residues accumulating in DNA with age. Incubation of the brain cells with human OGG1 represents the increase in the number of OGG1 sensitive sites with age. In neuronal DNA, the content of OGG1 sensitive sites between the young and adult ages has increased by 5.2-fold and between young and old ages by 7.4-fold. Closer observation of the data revealed that accumulations of OGG1 sensitive sites occurred continuously through adulthood and old age unlike the general damage due to overall strand breaks, which occurred mostly by adulthood itself (Table 2.2).

Similarly, the UDG sensitive sites in neurons at these three ages were assessed by the introduction of UDG, which removes uracil and creates AP sites that are cleaved to result in strand breaks during the alkali processing. When neurons were incubated with only buffer, increase of DNA migration in the tail was observed with age of the animal (Table 2.3). This increase in tail movement is taken as increased damage occurring due to aging. When the cells were incubated with buffer containing the UDG, there was a further increase in tail movement, which must be due to the presence of uracil residues accumulating in DNA with age. Essentially the



**Table 2.3** Measurement of UDG sensitive sites in young, adult and old rat brain neurons by FLARE™ comet assay. (Adapted from Swain and Subba Rao 2011)

Sample	Tail moment		Net amount of UDG sensitive sites
	Mean±SEM		
	Buffer	UDG	
Young neurons	14.96±1.70	22.77±2.15	7.81
Adult neurons	115.71±4.35	141.78±9.24 <sup>a***</sup>	26.07
Old neurons	134.70±5.91	185.34±10.55 <sup>b, c***</sup>	50.64

a and b are significantly different from the corresponding values at young; c is significantly different from the corresponding values at adult|

\*\*\**p*<0.001

enhanced tail movement due to the incubation of the neurons with UDG denotes the increase in the number of uracil or other UDG sensitive sites with age. Table 2.3 shows the actual fold increase of such sensitive sites with age in neurons. For example, in neuronal DNA, the content of UDG sensitive sites between the young and adult ages has increased 3.3-fold, and between young and old ages by 6.5-fold. Keen observation of the data suggests that accumulation of UDG sensitive sites in neurons is a gradual process with age (Table 2.3).

A striking feature appears to be that the damage accumulation occurs very rapidly during the first 6 months of life. A somewhat comparable result was noticed in a very early study carried out by Price et al. (1971). It is possible that this phenomenon of significant accumulation of DNA damage coupled with decreased DNA repair, particularly in a post-mitotic tissue, may have something to do with the timing of the attainment of reproductive maturity of the animal (Bernstein and Bernstein 1991). This, however, remains a speculation at this time. It is also possible that during this rapid growth and reproductive phase of the animal, the repair efficiency is not able to match with the accumulation of damage.

2.4 BER Pathway

BER is perhaps the most fundamental and ubiquitous DNA repair mechanism in all-higher organisms that depend on oxygen for the sustenance of life. This pathway has evolved to handle the numerous minor alterations—including spontaneous modification, oxidation, alkylation, deamination and loss of bases—that can occur in the structure of DNA as a result of cellular metabolic activity. This mode of repair is of particular importance in post-mitotic tissues such as those of the brain, where simple base modifications are more likely to occur than is major damage to DNA.

The core BER pathway requires the function of only four enzymes in the basic reaction steps to remove a damaged DNA base and replace it with the correct base. These proteins include a DNA glycosylase, an AP endonuclease or AP lyase, a DNA polymerase, and a DNA ligase. BER is initiated by a lesion-specific DNA



glycosylase (mono- or bi- functional) and completed by either of two sub-pathways: short-patch BER (SP-BER); a mechanism whereby only one nucleotide is replaced or long-patch BER (LP-BER); a mechanism whereby 2–13 nucleotides are replaced (Wilson 1998; Fortini et al. 1999; Fortini 2003; Almeida and Sobol 2007; Wilson and Bohr 2007; Hegde et al. 2008; Jeppesen et al. 2011). A currently accepted model for the core BER pathway reveals five distinct enzymatic steps for the repair of damaged bases.

1. Recognition and excision of the damage base,
2. Incision of the DNA adjacent to the resulting abasic site,
3. End cleaning of the DNA termini to produce a 3'-hydroxyl group (3'-OH) and a 5'-phosphate group (5'-P),
4. Repair synthesis, and
5. DNA ligation to seal the nick.

(i) The initiation of BER is performed by a specific DNA glycosylase that recognizes and excises a specific base by catalyzing hydrolysis of the N-glycosylic bond resulting in an AP site. Depending on the types of damaged base, DNA glycosylase can be either monofunctional (such as UDG, has only the glycosylase activity) or bifunctional (such as OGG1 and NEILS, have an intrinsic 3'AP lyase activity in addition to the glycosylase activity).

(ii) The second step consists of incision of DNA strand at 5' to the AP site by major apurinic endonuclease 1 (APE1), leaving a 5'-deoxyribose phosphate (5'-dRP) and 3'-OH group (Demple et al. 1991; Robson et al. 1992; Seki et al. 1992; Demple and Sung 2005). The bifunctional DNA glycosylase incises the DNA strand at 3' to the AP site via  $\beta$ - or  $\beta\delta$ -elimination, leaving a DNA SSB with a 3'-phospho- $\alpha,\beta$ -unsaturated aldehyde (3'-PUA) or a 3'-phosphate (3'-P), respectively (Hazra et al. 2002, 2007; Pascucci et al. 2002; Dou et al. 2003).

(iii) The third step in BER is end cleaning of obstructive 3'- and 5'-termini to generate the 3'-OH and 5'-P groups in the gap at the strand break, which is the favorable substrate for DNA pol  $\beta$ . Pol  $\beta$  is a 39 kDa single polypeptide comprising 335 amino acid residues. Both the rat and human enzymes were cloned 15 yrs ago and extensively studied over the years by Wilson and his group (SenGupta et al. 1986) and by Matsukage's group (Date et al. 1988). The structural, catalytic and physiological aspects of pol  $\beta$  have been the subjects of two elegant reviews by Wilson and his associates (Wilson 1998; Idriss et al. 2002). The 5'-dRP group is removed by pol  $\beta$ , by its dRP lyase activity (Matsumoto and Kim 1995; Prasad et al. 1998; Allinson et al. 2001). On the other hand, APE1 removes the 3'-PUA group generated by  $\beta$ -elimination via its 3'-phosphodiesterase activity (Suh et al. 1997; Izumi et al. 2000). The 3'-P group generated by  $\beta\delta$ -elimination is a poor substrate for APE1 (Wilson 2003), so such blocking groups are excised primarily by phosphatase activity of polynucleotide kinase 3'-phosphatase (PNKP) (Jilani et al. 1999; Dobson and Allinson 2006).

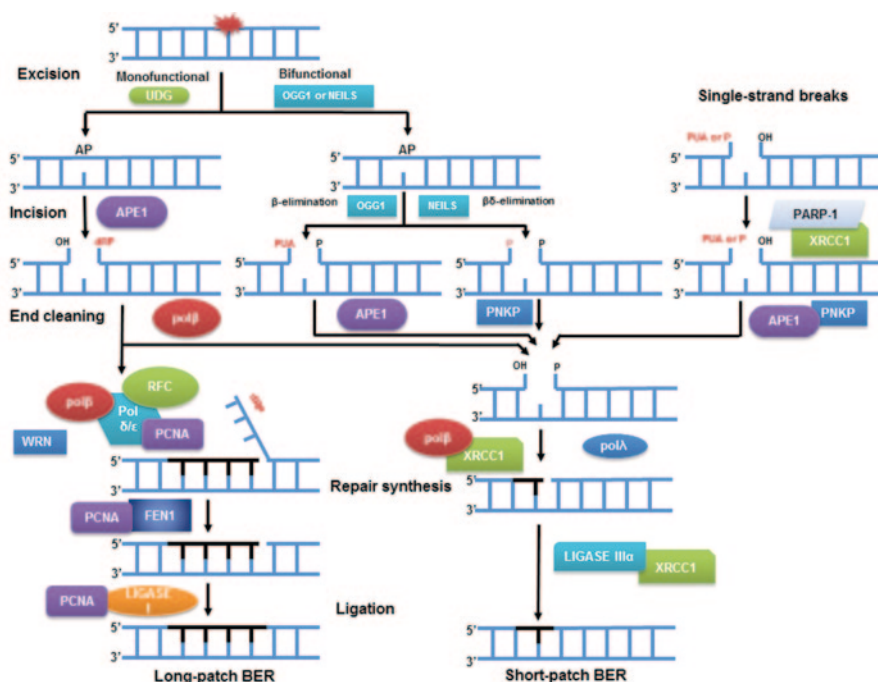
(iv) In this step, the repair synthesis to replace nucleotide(s), can proceed by one of two sub- pathways, SP-BER or LP-BER., when the 5'-dRP can be efficiently removed by pol  $\beta$  in the previous step (iii), SP-BER is favored. In SP-BER, pol

$\beta$  fills up one nucleotide gap (Sobol et al. 1996), and also interacts with X-ray cross-complementing 1 (XRCC1), a scaffold protein involved in prompting SP-BER by interacting with other proteins (Kubota et al. 1996; Dianova et al. 2004). The majority of BER events are currently thought to occur via SP-BER. LP-BER is initiated in cases where the 5'-dRP group is refractory to the pol  $\beta$  AP lyase activity (for example, reduced AP site) (Podlutzky et al. 2001), the repair synthesis would nevertheless continue but in a strand displacement manner. In LP-BER, the repair synthesis of 2–13 nucleotides is performed by pol  $\beta$ , and/or pol  $\delta/\epsilon$  coupled with proliferating cell nuclear antigen (PCNA) incorporation with loading factor RFC. The resulting 5'-flap structure formed during repair synthesis is removed by the flap endonuclease 1 (FEN1), the activity of which is stimulated by PCNA (Klungland and Lindahl 1997; Fortini et al. 1998; Stucki et al. 1998; Gary et al. 1999). In addition, a number of accessory proteins have been reported to participate in and/or stimulate BER, for example, Poly (ADP-ribose) polymerase-1 (PARP-1) stimulates the strand displacement synthesis of pol  $\beta$  in the presence of FEN1 in LP-BER (Prasad et al. 2001). The Werner syndrome protein helicase (WRN) is also observed to stimulate strand displacement activities of pol  $\beta$  (Harrigan et al. 2003).

(v) The final step in BER is ligation to seal the nick containing a 3'-OH group and a 5'-P group. In SP-BER, the ligation of the final nick is performed by DNA ligase III $\alpha$  (LIGIII  $\alpha$ ) in association with scaffold protein XRCC1 (Caldecott et al. 1994; Cappelli et al. 1997; Tomkinson et al. 2001). In LP-BER, ligation is performed by DNA ligase I (LIGI) with the physical association with PCNA, which helps to stimulate the effective ligation (Levin et al. 2000; Tom et al. 2001; Tomkinson et al. 2001).

SSBs resulting from a variety of causes like ionizing radiation, ROS etc., can also be repaired through BER. Sometimes if the break has a 3'-P or 3'-PUA and 5'-OH then the phosphorylation of 5'-OH and removal of phosphate from 3'-end or removal of 3'-PUA becomes necessary before the break is filled up through BER pathway. This operation is achieved by the enzymes PNKP or APE1. Both enzymes thus carry out important functions of converting the ends of a break in DNA into a repairable mode. If these preparative changes are not taken fast enough, then there is the possibility of undesirable recombination events taking place. In order to avoid this eventuality, PARP-1 provides protection to the DNA SSBs by binding to them (Le Rhun et al. 1998; Ziegler and Oei 2001; Chalmers 2004). There is also a report that PARP-1 interacts with XRCC1 and this interaction accelerates the recruitment of repair proteins involved in BER pathway (Mackey et al. 1999). XRCC1 also stimulates the DNA kinase and DNA phosphatase activities of PNK at damaged DNA termini and thereby accelerates the overall repair process (Whitehouse et al. 2001).

Finally, a newly discovered polymerase is claiming its entry as a possible participant in the BER pathway- DNA polymerase  $\lambda$  (pol  $\lambda$ ). Pol  $\lambda$  is a recently described eukaryotic DNA polymerase belonging to Pol X family. It is the closest homologue to pol  $\beta$ , sharing 32 % identity at the protein level. Based on three-dimensional structure modeling, Pol  $\lambda$  is predicted to have a pol  $\beta$  like core formed by two domains: a 31 kDa polymerase domain and 8 kDa domain (Garcia-Diaz et al. 2000;

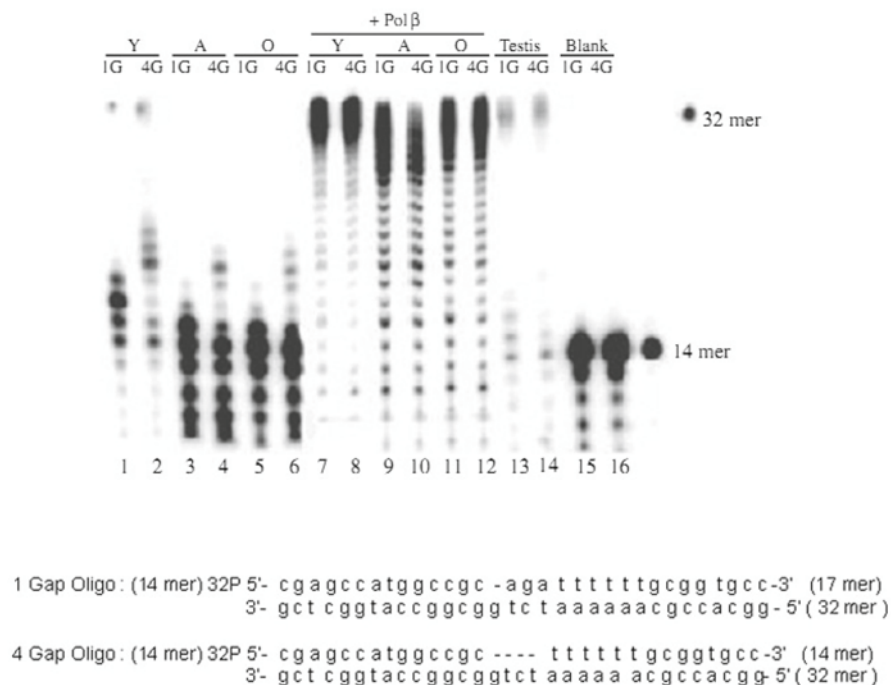


**Fig. 2.3** Schematic representation of the mammalian base excision repair and single-strand break repair pathway

DeRose et al. 2003). Recently, Gap-filling DNA synthesis and dRP lyase activities of pol  $\lambda$  have been demonstrated in vitro, suggesting its participation in BER (Garcia-Diaz et al. 2001; Braithwaite et al. 2005). Figure 2.3 shows the BER pathway depicted on the basis of latest information available.

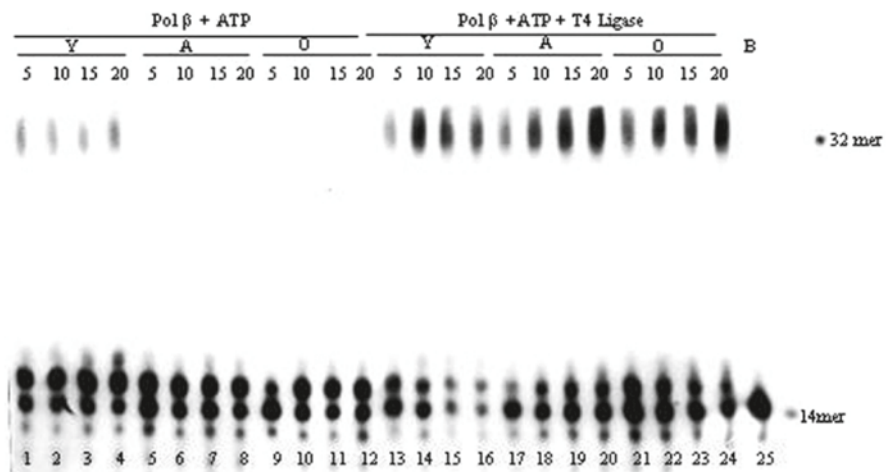
## 2.5 BER and Brain

In the BER pathway, the actual substrate for pol  $\beta$  is a DNA duplex with one of the strands with a baseless site, which is eventually converted into a gap. Thus, a gap DNA is the most natural substrate for pol  $\beta$  to insert the correct nucleotide using the other strand as the template. In view of this, synthetic oligo duplexes (32-mers) with one or four nucleotide gap in one of the strands were used as substrates and the gap filling activity in young, adult and old neuronal extracts was measured (Krishna et al. 2005). Gap repair involves two steps: the filling of the gap by the addition of the required number of nucleotides followed by the ligation with the 5' phosphorylated downstream primer. This repair process, if completed properly should give a radioactive spot on the sequencing gel corresponding to the 32-mer.



**Fig. 2.4** Gap repair activity in 'young,' 'adult' and 'old' neuronal extracts and supplemented with recombinant pure rat liver pol  $\beta$  with 5'-PO<sub>4</sub> on the downstream primer. Lanes 1–6 neuronal extracts from young brain (Y, 5 days postnatal), adult brain (A, 6 months) old brain (O,  $\geq 2$  years). Lanes 7–12 neuronal extracts supplemented with one unit of pol  $\beta$ . Lanes 13 and 14 are with testis extracts alone as positive control. Lanes 15 and 16 are without any neuronal extracts (enzyme blanks). The mobility of labeled standard, 14-mer and 32-mer is also shown. Lanes 1, 3, 5, 7, 9, 11, 13, 15 are with the one-gap substrate (1G) while lanes 2, 4, 6, 8, 10, 12, 14, 16 are with the four-gap substrate (4G). The oligo duplexes with one and four nucleotide gaps used in the study are also shown. As can be seen one of the strands has a gap of either 1 or 4 nucleotides. These strands are <sup>32</sup>P labeled on 5' end for the subsequent identification on the sequencing gel followed by autoradiography. Furthermore, in either case, the downstream primer after the gap is phosphorylated on 5' end with non-radioactive phosphate before annealing. (Adapted from Rao 2007)

However, it is seen that only addition of nucleotide has occurred with adult and old neuronal extracts. In the young, addition of nucleotides was seen and ligation to downstream primer also occurred although quite feebly. On the other hand, when the extracts were supplemented with pol  $\beta$ , addition of nucleotides occurred all the way to extend the upstream primer to a 32-mer apparently in a distributive strand displacement manner (Fig. 2.4). On the other hand, when low amounts of pol  $\beta$  were added, addition of just the required number of nucleotides occurred. Even then ligation was achieved only in young extracts, and no ligation could be visualized in adult and old. Finally efficient gap filling followed by ligation, that is complete gap repair, was achieved and for this to happen, conditions required are the presence of



**Fig. 2.5** Restoration of the gap repair activity in adult and old rat neuronal extracts when supplemented with limited amounts of pol β (0.2 units) and 20 units of T<sub>4</sub> DNA ligase. All the experimental details and notations are as in Fig. 2.4. Only one gap duplex was used as substrate

5'-PO<sub>4</sub> on the downstream primer, and supplementation of aging neuronal extracts with both pol β and DNA ligase (Fig. 2.5). These studies thus demonstrated that aging neurons are unable to affect BER, due to deficiency of pol β and DNA ligase and fortifying the neuronal extracts from aged animals with these two factors can restore the lost BER activity.

While pol β together with DNA ligase in the case of DNA gap repair could restore the repair activities when supplemented to neuronal extracts from old animals, it is possible that some other polymerases present in neurons may also be functioning in the BER pathway. In recent times many new DNA polymerases, generally error prone and capable of carrying out a variety of other tasks have been discovered (Hubscher et al. 2002; Bebenek and Kunkel 2004). One of them is pol λ belonging to the same family as pol β does, the X-family. The structure and sequence homology of pol λ are similar to pol β and in particular, pol λ contains all the critical residues involved in the DNA binding, nucleotide binding and selection, and catalysis of DNA polymerization, that are conserved in pol β and other DNA polymerases belonging to X-family (Garcia-Diaz et al. 2000), and therefore, emerging as a candidate that could help/substitute pol β in its role in BER (Garcia-Diaz et al. 2005). However, there is no concrete information available about the levels of these novel DNA polymerases in brain. In a preliminary Western blot analysis of pol λ in rodent brain regions, we have noticed the presence of pol λ in brain extracts. Similarly, immunoprecipitated pol λ from brain extract is able to fill one nucleotide gap in one of the strands of DNA oligoduplexes (Swain et al. unpublished observations). A comprehensive study to examine the status of many of these newly discovered DNA polymerases in brain is warranted.

## 2.6 Conclusions

DNA damage is an extremely common event in all living cells. Both intrinsic as well as extrinsic factors cause the damage. ROS cause a major form of damage that could be deleterious, if not repaired. As organisms age, their DNA repair capacity decreases and this coupled with accumulating damage to DNA may eventually lead to breakdown of the cellular machinery culminating in disease and finally death. Among the various pathways of DNA repair, BER vested with the responsibility of correcting the simple alterations in DNA structure like base modifications and AP sites, essentially resulting from the active metabolism within the cell, can perhaps be viewed as a fundamental housekeeping repair mechanism to safeguard the genomic integrity not only in a post-mitotic organ like brain but in the rest of the body as well. The activity of some important components of this pathway, like pol  $\beta$  and DNA ligase, is compromised in brain cells with age and may be taken as molecular markers for the genomic stability and process of senescence. Thus, any inherited mutational vulnerability in this pathway could display telling effects on the process of aging and associated disorders.

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## References

- Alexander P (1967) The role of DNA lesions in the processes leading to aging in mice. *Symp Soc Exp Biol* 21:29–50
- Allinson SL, Dianova II, Dianov GL (2001) DNA polymerase  $\beta$  is the major dRP lyase involved in repair of oxidative base lesions in DNA by mammalian cell extracts. *EMBO J* 20:6919–6926
- Almeida KH, Sobol RW (2007) A unified view of base excision repair: lesion-dependent protein complexes regulated by post-translational modification. *DNA Repair (Amst)* 6:695–711
- Bandyopadhyay U, Das D, Banerjee RK (1999) Reactive oxygen species: Oxidative damage and pathogenesis. *Curr Sci* 77:658–666
- Barnes DE, Lindahl T (2004) Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* 38:445–476
- Barzilai A (2007) The contribution of the DNA damage response to neuronal viability. *Antioxid Redox Signal* 9:211–218
- Barzilai A (2010) DNA damage, neuronal and glial cell death and neurodegeneration. *Apoptosis* 15:1371–1381
- Bebenek K, Kunkel TA (2004) Functions of DNA polymerases. *Adv Protein Chem* 69:137–165
- Bernstein C, Bernstein H (1991) Aging, sex, and DNA repair. Academic, San Diego
- Braithwaite EK, Prasad R, Shock DD, Hou EW, Beard WA, Wilson SH (2005) DNA polymerase lambda mediates a back-up base excision repair activity in extracts of mouse embryonic fibroblasts. *J Biol Chem* 280:18469–18475
- Cadet J, Delatour T, Douki T, Gasparutto D, Pouget JP, Ravanat JL, Sauvaigo S (1999) Hydroxyl radicals and DNA base damage. *Mutat Res* 424:9–21
- Caldecott KW, Mckeown CK, Tucker JD, Ljungquist S, Thompson LH (1994) An interaction between the mammalian DNA-mepair protein Xrcc1 and DNA Ligase-III. *Mol Cell Biol* 14:68–76



- Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, Frosina G (1997) Involvement of XRCC1 and DNA ligase III gene products in DNA base excision repair. *J Biol Chem* 272:23970–23975
- Chalmers AJ (2004) Poly(ADP-ribose) polymerase-1 and ionizing radiation: sensor, signalling and therapeutic target. *Clin Oncol (R Coll Radiol)* 16:29–39
- Clark DD, Sokoloff L (1999) Circulation and energy metabolism of the brain. In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD (eds) *Basic neurochemistry. Molecular, cellular and medical aspects*, 6th edn. Lippincott-Raven, Philadelphia, pp. 637–670
- Collins AR (2009) Investigating oxidative DNA damage and its repair using the comet assay. *Mutat Res* 681:24–32
- Cooke MS, Evans MD, Dizdaroglu M, Lunec J (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* 17:1195–1214
- Date T, Yamaguchi M, Hirose F, Nishimoto Y, Tanihara K, Matsukage A (1988) Expression of active rat DNA polymerase beta in *Escherichia coli*. *Biochemistry (Mosc)* 27:2983–2990
- Demple B, Harrison L (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annu Rev Biochem* 63:915–948
- Demple B, Sung JS (2005) Molecular and biological roles of Ape1 protein in mammalian base excision repair. *DNA Repair (Amst)* 4:1442–1449
- Demple B, Herman T, Chen DS (1991) Cloning and expression of APE, the cDNA encoding the major human apurinic endonuclease: definition of a family of DNA repair enzymes. *Proc Natl Acad Sci USA* 88:11450–11454
- DeRose EF, Kirby TW, Mueller GA, Bebenek K, Garcia-Diaz M, Blanco L, Kunkel TA, London RE (2003) Solution structure of the lyase domain of human DNA polymerase lambda. *Biochemistry (Mosc)* 42:9564–9574
- Dianova II, Sleeth KM, Allinson SL, Parsons JL, Breslin C, Caldecott KW, Dianov GL (2004) XRCC1–DNA polymerase  $\beta$  interaction is required for efficient base excision repair. *Nucleic Acids Res* 32:2550–2555
- Dobson CJ, Allinson SL (2006) The phosphatase activity of mammalian polynucleotide kinase takes precedence over its kinase activity in repair of single strand breaks. *Nucleic Acids Res* 34:2230–2237
- Dou H, Mitra S, Hazra TK (2003) Repair of oxidized bases in DNA bubble structures by human DNA glycosylases NEIL1 and NEIL2. *J Biol Chem* 278:49679–49684
- Duncan BK, Miller JH (1980) Mutagenic deamination of cytosine residues in DNA. *Nature* 287:560–561
- Ehrlich M, Zhang XY, Inamdar NM (1990) Spontaneous deamination of cytosine and 5-methylcytosine residues in DNA and replacement of 5-methylcytosine residues with cytosine residues. *Mutat Res* 238:277–286
- Evans MD, Dizdaroglu M, Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. *Mutat Res* 567:1–61
- Fishel ML, Vasko MR, Kelley MR (2007) DNA repair in neurons: so if they don't divide what's to repair? *Mutat Res* 614:24–36
- Fortini P (2003) The base excision repair: mechanisms and its relevance for cancer susceptibility. *Biochimie* 85:1053–1071
- Fortini P, Pascucci B, Parlanti E, Sobol RW, Wilson SH, Dogliotti E (1998) Different DNA polymerases are involved in the short- and long-patch base excision repair in mammalian cells. *Biochemistry (Mosc)* 37:3575–3580
- Fortini P, Parlanti E, Sidorkina OM, Laval J, Dogliotti E (1999) The type of DNA glycosylase determines the base excision repair pathway in mammalian cells. *J Biol Chem* 274:15230–15236
- Garcia-Diaz M, Dominguez O, Lopez-Fernandez LA, de Lera LT, Saniger ML, Ruiz JF, Parraga M, Garcia-Ortiz MJ, Kirchhoff T, del Mazo J, Bernad A, Blanco L (2000) DNA polymerase lambda (Pol lambda), a novel eukaryotic DNA polymerase with a potential role in meiosis. *J Mol Biol* 301:851–867



- Garcia-Diaz M, Bebenek K, Kunkel TA, Blanco L (2001) Identification of an intrinsic 5'-deoxy-ribose-5-phosphate lyase activity in human DNA polymerase lambda: a possible role in base excision repair. *J Biol Chem* 276:34659–34663
- Garcia-Diaz M, Bebenek K, Gao G, Pedersen LC, London RE, Kunkel TA (2005) Structure-function studies of DNA polymerase lambda. *DNA Repair (Amst)* 4:1358–1367
- Gary R, Kim K, Cornelius HL, Park MS, Matsumoto Y (1999) Proliferating cell nuclear antigen facilitates excision in long-patch base excision repair. *J Biol Chem* 274:4354–4363
- Halliwell B (1992) Reactive oxygen species and the central nervous system. *J Neurochem* 59:1609–1623
- Harrigan JA, Opresko PL, von Kobbe C, Kedar PS, Prasad R, Wilson SH, Bohr VA (2003) The Werner syndrome protein stimulates DNA polymerase beta strand displacement synthesis via its helicase activity. *J Biol Chem* 278:22686–22695
- Hazra TK, Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M, Mitra S (2002) Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proc Natl Acad Sci U S A* 99:3523–3528
- Hazra TK, Das A, Das S, Choudhury S, Kow YW, Roy R (2007) Oxidative DNA damage repair in mammalian cells: a new perspective. *DNA Repair (Amst)* 6:470–480
- Hegde ML, Hazra TK, Mitra S (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. *Cell Res* 18:27–47
- Hubscher U, Maga G, Spadari S (2002) Eukaryotic DNA polymerases. *Annu Rev Biochem* 71:133–163
- Idriss HT, Al-Assar O, Wilson SH (2002) DNA polymerase beta. *Int J Biochem Cell Biol* 34:321–324
- Izumi T, Hazra TK, Boldogh I, Tomkinson AE, Park MS, Ikeda S, Mitra S (2000) Requirement for human AP endonuclease 1 for repair of 3'-blocking damage at DNA single-strand breaks induced by reactive oxygen species. *Carcinogenesis* 21:1329–1334
- Jeppesen DK, Bohr VA, Stevnsner T (2011) DNA repair deficiency in neurodegeneration. *Prog Neurobiol* 94:166–200
- Jilani A, Ramotar D, Slack C, Ong C, Yang XM, Scherer SW, Lasko DD (1999) Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3'-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. *J Biol Chem* 274:24176–24186
- Klungland A, Lindahl T (1997) Second pathway for completion of human DNA base excision-repair: reconstitution with purified proteins and requirement for DNase IV (FEN1). *EMBO J* 16:3341–3348
- Krishna TH, Mahipal S, Sudhakar A, Sugimoto H, Kalluri R, Rao KS (2005) Reduced DNA gap repair in aging rat neuronal extracts and its restoration by DNA polymerase beta and DNA-ligase. *J Neurochem* 92:818–823
- Kubota Y, Nash RA, Klungland A, Schar P, Barnes DE, Lindahl T (1996) Reconstitution of DNA base excision-repair with purified human proteins: Interaction between DNA polymerase beta and the XRCC1 protein. *EMBO J* 15:6662–6670
- Le Rhun Y, Kirkland JB, Shah GM (1998) Cellular responses to DNA damage in the absence of Poly(ADP-ribose) polymerase. *Biochem Biophys Res Commun* 245:1–10
- Lemay M, Wood KA (1999) Detection of DNA damage and identification of UV-induced photo-products using the CometAssay kit. *Biotechniques* 27:846–851
- Levin DS, McKenna AE, Motycka TA, Matsumoto Y, Tomkinson AE (2000) Interaction between PCNA and DNA ligase I is critical for joining of Okazaki fragments and long-patch base-excision repair. *Curr Biol* 10:919–922
- Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature* 362:709–715
- Lindahl T, Nyberg B (1972) Rate of depurination of native deoxyribonucleic acid. *Biochemistry (Mosc)* 11:3610–3618
- Lindahl T, Karlstrom O (1973) Heat-induced depyrimidination of deoxyribonucleic acid in neutral solution. *Biochemistry (Mosc)* 12:5151–5154

- Lindahl T, Nyberg B (1974) Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry (Mosc)* 13:3405–3410
- Loeb LA (1989) Endogenous carcinogenesis: molecular oncology into the twenty-first century—presidential address. *Cancer Res* 49:5489–5496
- Loeb LA, Kunkel TA (1982) Fidelity of DNA synthesis. *Annu Rev Biochem* 51:429–457
- Mackey ZB, Niedergang C, Ménissier-de Murcia J, Leppard J, Au K, Chen J, de Murcia G, Tomkinson AE (1999) DNA ligase III is recruited to DNA strand breaks by a zinc finger motif homologous to that of poly (ADP-ribose) polymerase. *J Biol Chem* 274:21679–21687
- Mandavilli BS, Rao KS (1996a) Accumulation of DNA damage in aging neurons occurs through a mechanism other than apoptosis. *J Neurochem* 67:1559–1565
- Mandavilli BS, Rao KS (1996b) Neurons in the cerebral cortex are most susceptible to DNA-damage in aging rat brain. *Biochem Mol Biol Int* 40:507–514
- Matsumoto Y, Kim K (1995) Excision of deoxyribose phosphate residues by DNA polymerase beta during DNA repair. *Science* 269:699–702
- Ostling O, Johanson KJ (1984) Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. *Biochem Biophys Res Commun* 123:291–298
- Pascucci B, Maga G, Hubscher U, Bjoras M, Seeberg E, Hickson ID, Villani G, Giordano C, Cellai L, Dogliotti E (2002) Reconstitution of the base excision repair pathway for 7,8-dihydro-8-oxoguanine with purified human proteins. *Nucleic Acids Res* 30:2124–2130
- Podlutzky AJ, Dianova, II, Podust VN, Bohr VA, Dianov GL (2001) Human DNA polymerase beta initiates DNA synthesis during long-patch repair of reduced AP sites in DNA. *EMBO J* 20:1477–1482
- Prasad R, Beard WA, Strauss PR, Wilson SH (1998) Human DNA polymerase  $\beta$  deoxyribose phosphate lyase. *J Biol Chem* 273:15263–15270
- Prasad R, Lavrik OI, Kim SJ, Kedar P, Yang XP, Vande Berg BJ, Wilson SH (2001) DNA polymerase beta -mediated long patch base excision repair. Poly(ADP-ribose) polymerase-1 stimulates strand displacement DNA synthesis. *J Biol Chem* 276:32411–32414
- Price GB, Modak SP, Makinodan T (1971) Age-associated changes in the DNA of mouse tissue. *Science* 171:917–920
- Rao KS (1993) Genomic damage and its repair in young and aging brain. *Mol Neurobiol* 7:23–48
- Rao KS (2007) DNA repair in aging rat neurons. *Neuroscience* 145:1330–1340
- Richter C, Park JW, Ames BN (1988) Normal Oxidative Damage to Mitochondrial and Nuclear-DNA Is Extensive. *Proc Natl Acad Sci U S A* 85:6465–6467
- Riley PA (1994) Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol* 65:27–33
- Robson CN, Hochhauser D, Craig R, Rack K, Buckle VJ, Hickson ID (1992) Structure of the human DNA repair gene HAP1 and its localisation to chromosome 14q 11.2–12. *Nucleic Acids Res* 20:4417–4421
- Seki S, Hatsushika M, Watanabe S, Akiyama K, Nagao K, Tsutsui K (1992) cDNA cloning, sequencing, expression and possible domain structure of human APEX nuclease homologous to Escherichia coli exonuclease III. *Biochim Biophys Acta* 1131:287–299
- SenGupta D, Zmudzka B, Kumar P, Cobianchi F, Skowronski J, Wilson S (1986) Sequence of human DNA polymerase [beta] mRNA obtained through cDNA cloning. *Biochem Biophys Res Commun* 136:341–347
- Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 175:184–191
- Sobol RW, Horton JK, Kühn R, Gu H, Singhal RK, Prasad R, Rajewsky K, Wilson SH (1996) Requirement of mammalian DNA polymerase- $\beta$  in base-excision repair. *Nature* 379:183–186
- Stucki M, Pascucci B, Parlanti E, Fortini P, Wilson SH, Hubscher U, Dogliotti E (1998) Mammalian base excision repair by DNA polymerases delta and epsilon. *Oncogene* 17:835–843
- Subbarao K, Loeb L (1992) DNA damage and repair in brain: relationship to aging. *Mutation Research/DNAging* 275:317–329
- Suh D, Wilson DM, Povirk LF (1997) 3'-Phosphodiesterase activity of human apurinic/apyrimidinic endonuclease at DNA double-strand break ends. *Nucleic Acids Res* 25:2495–2500

- Swain U, Subba Rao K (2011) Study of DNA damage via the comet assay and base excision repair activities in rat brain neurons and astrocytes during aging. *Mech Aging Dev* 132:374–381
- Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen* 35:206–221
- Tom S, Henricksen LA, Park MS, Bambara RA (2001) DNA ligase I and proliferating cell nuclear antigen form a functional complex. *J Biol Chem* 276:24817–24825
- Tomkinson AE, Chen L, Dong Z, Leppard JB, Levin DS, Mackey ZB, Motycka TA (2001) Completion of base excision repair by mammalian DNA ligases. *Prog Nucleic Acid Res Mol Biol* 68:151–164
- Whitehouse CJ, Taylor RM, Thistlethwaite A, Zhang H, Karimi-Busheri F, Lasko DD, Weinfeld M, Caldecott KW (2001) XRCC1 stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. *Cell* 104:107–117
- Wilson SH (1998) Mammalian base excision repair and DNA polymerase beta. *Mutat Res* 407:203–215
- Wilson DM, 3rd (2003) Properties of and substrate determinants for the exonuclease activity of human apurinic endonuclease Ape1. *J Mol Biol* 330:1027–1037
- Wilson DM, 3rd, Sofinowski TM, McNeill DR (2003) Repair mechanisms for oxidative DNA damage. *Front Biosci* 8:d963–981
- Wilson DM, 3rd, Bohr VA (2007) The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair (Amst)* 6:544–559
- Ziegler M, Oei SL (2001) A cellular survival switch: poly(ADP-ribosylation) stimulates DNA repair and silences transcription. *Bioessays* 23:543–548



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