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DNA-Repair and Brain Aging: The Importance of Base Excision Repair and DNA Polymerase β

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The article reviews briefly the recent information available to point out the close relation between DNA-repair and aging process. Various types of structural alterations that are known to occur in genomic DNA of mammalian cells and the DNA-repair systems that operate to counteract such DNA damage are outlined with special emphasis on mammalian brain. The importance of the Base Excision Repair mode of repair and that of DNA-polymerase b in neuronal cells is highlighted. Evidence that has accumulated in recent past from author's as well as other laboratories indicates: (1) Base Excision Repair is markedly low and this is possibly due to decreased catalytic activity of DNA-Polymerase β, a crucial component of Base Excision Repair pathway; (2) In certain *in vitro* conditions with a model synthetic substrate, the lost pol β activity could be restored through supplementation of pure DNA-polymerase β to the neuronal extracts. The possible implications of these observations in terms of future directions of research in this area are projected.

Key Words: DNA-damage, DNA-repair, Life span, Aging, Brain, neurons, Base Excision Repair, DNA-polymerase β

Introduction

One of the most astonishing facets of evolution of living organisms is the manner in which they have evolved a mechanism to maintain their own genomic integrity. Thus at any given level of organic evolution, the species are faced with two opposing forces. One of them is to continue the propagation of that species without permitting any major alteration in the genomic characteristics while the other is to permit or undergo such changes so as to make the species better equipped to overcome the environmental stresses and hazards. Implicit in these phenomena is the fact that even under the normal conditions and in the absence of any environmental insult, genomic DNA's structural integrity is under constant threat and there was the necessity to develop machinery to counteract the inevitable damage that occurs to DNA under normal metabolic conditions. This machinery is the one, which is called to day the DNA-repair system, which out of necessity, is conserved as well as evolved along with the living world.

Since DNA-repair systems are essential for the maintenance of genomic integrity, any insufficiency in these systems can be expected to cause abnormal situations like birth defects, increased risk of cancer and premature death. Indeed extensive investigations during the past several years on this subject have revealed that genomic instability (poor DNA-repair potential) has a tight correlation with the phenomena mentioned above (for reviews please see Kirkwood & Austad 2000, Hoeijmakers 2001). With the original findings of Alexander (1967) that DNA repair is at a low key once the cells are differentiated into 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. Nevertheless the information is so scanty as compared to the available knowledge of DNA-repair pathways in other mammalian tissues and

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lower organism like *E.coli* (reviewed in Rao 1993). However, it was realized soon that the terminally differentiated nature of brain cells offers a good model for studying the accumulation of genomic damage during the life span of a given animal.

It is therefore, the purpose of this article: (1) To review, very briefly, the information available to suggest a fundamental role for DNA-repair systems in the process of aging; (2) Then, to present, with a special emphasis on brain tissue, an outline of the various types of structural alterations that are known to occur in genomic DNA of mammalian cells and the DNA-repair pathways that operate to counteract such DNA damage; (3) To present recently accumulated evidence to suggest that Base Excision Repair (BER) and DNA-polymerase β (pol β), a key player in the BER pathway, play a crucial role in the maintenance of the structural integrity of genomic DNA in brain and that BER is markedly decreased in aging brain; and (4) To present a critical appraisal of the possible consequences of such attenuated BER and stress the need to find possible ways that could restore the lost BER activity in aging brain.

DNA-repair and Aging

The relationship between DNA-repair potential and the lifespan of any given species has been the subject of study since a long time. In a classical paper by Hart and Setlow (1974), it was postulated that an animal's ability to repair certain types of DNA-damage (e.g. uv-induced thymidine dimers) has a direct correlation to the lifespan of that species. For example, humans repair uv damaged DNA more quickly and efficiently than rat or such other animals with shorter life span. This earlier observation has been confirmed by some others over the years (Smith-Sonneborn 1979, Wei et al. 1993, Cortopassi & Wang 1996, Zahn et al. 2000, de Boer et al. 2002). Many other workers have looked for changes in DNA-repair capacity as well as of accumulation of DNA-damage at different points of the lifespan of various experimental models. This subject is so enormous and as such it is beyond the scope of this article to go into details of these studies. Suffice it to say that after a period of conflicting reports, it is now generally accepted that decrease in DNA-repair capacity and increase in DNA-damage do occur with age in several experimental models and comprehensive reviews/reports have appeared on this topic (Bernstein & Bernstein 1991, Wei et al. 1993, Rao 1998, Finkel & Holbrook 2000, Walter et al. 2001, Burkle 2001, Mohaghegh & Hickson 2001, Wolf et al. 2002, de Boer et al. 2002).

The most compelling evidence that the aging phenomenon has a genetic link has come from molecular genetic studies. Several genes (the present number is around 120) both in mammalian and non-mammalian species are found to have telling affect on the lifespan. Mutations in some of these genes are seen to increase the lifespan while in other genes decrease the lifespan (Finkel & Holbrook 2000, for a list of all those genes affecting the aging process and the lifespan, the reader is referred to the genes/interventions web site of Science's sage ke, http://sageke.sciencemag.org/cgi/genesdb).

The Uniqueness of Brain Cells

Mammalian brain consists of two major types of cells, the neurons and glia. Mature neurons are essentially post-mitotic and lack the capacity of proliferation whereas a small portion of the glial cells are known to be undergoing replication especially in response to stress or damage (Korr 1980, Ridet et al. 1997). Thus most of the cells in adult brain can be considered as non-dividing. Further, central nervous system is one of the earliest systems to develop and differentiate in almost all the species. It would therefore follow that a neuronal cell in brain of any species, at any given point of time, is virtually as old as the animal itself. Further, it is also known that a neuron is one of the most active cells metabolically in the body. Gene expression is 2 to 3 fold higher in brain cells as compared to the cells in other tissues. Also, more percentage of genomic DNA is expressed in neurons as opposed to a mere 2-5% in other parts of the body (Tobin 1994). All this points out the fact that brain cells are non-dividing but metabolically highly active. This situation also makes the brain tissue one of the most vulnerable organs in terms of the damage to cellular DNA due to various endogenous factors arising out of the high metabolic activity in these cells.

Types of DNA Damage

A plethora of alterations in the native structure of DNA can occur in the cell both due to external factors like radiation, toxic chemical substances in diet etc., and internal factors like intermediates and

products of normal metabolism itself. 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 shown in figures 1 and 2. Spontaneous damage can result in loss of bases; modification of bases, mismatched base pairs, strand breaks and also changes in the sequence of bases. Alterations in DNA structure could also occur due to environmental factors mentioned above causing strand breaks, cross links, formation of bulky adducts and oxidative damage.

The frequency with which the DNA damaging events described above occurs in a mammalian cell has been examined by a number of workers and this information is summarized in table 1.

Work from this laboratory, using two biochemical strategies, has demonstrated that neurons from different regions of rat brain harbor both SSB (single strand breaks) and DSB (double strand breaks) in their nuclear DNA and this strand breaks increase with age of the animal (Mandavilli & Rao 1996, 1996a). These data are summarized in table 2. Further, these studies also revealed an increase in the AP (apurinic/apyrimidinic) and alkali labile sites with age in the rat cerebral cortex (unpublished observations in author's lab).

As can be seen in table 2, the number of SSBs increases in neurons with age. There are 7,400 breaks

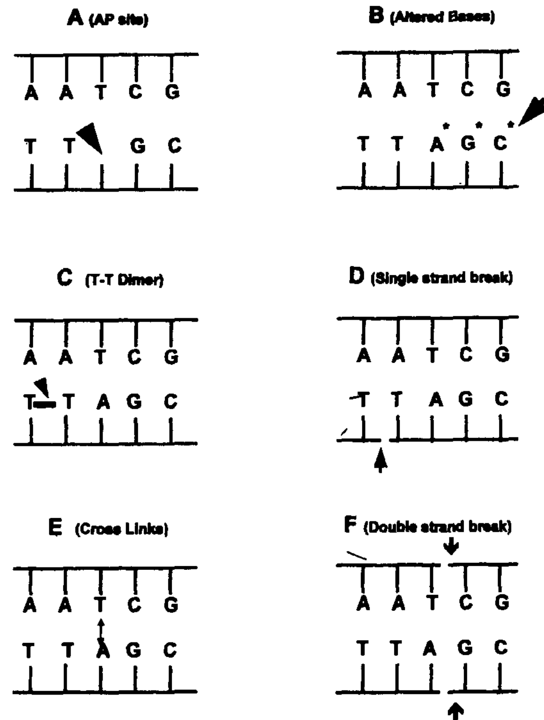

Figure 1 Different forms of DNA damage that could occur due to various endogenous and/or exogenous factors *A*, apurinic/apyrimidinic site (AP site); *B*, altered / modified bases that may result in mismatches; C, thymidine dimer, a major photoproduct formed owing to UV irradiation (250-260 nm); D, single-strand break, one of the most frequent consequential end points of various damages; E, cross-links formed between two strands of DNA (interstrand). Sometimes the cross-links are between DNA and the surrounding proteins; F, double-strand breaks - an occasional end point of oxidative/ionizing radiation induced damage

Table 1 *Approximate Frequeny of Occurrence of Various Types of DNA Damage in Mammalian Cells**

Type of damage	Events per day / cell	Reference
Depurination	10,000	Lindahl & Nyberg 1972
Depyrimidination	500	Lindahl & Karlstrom 1973
Deamination	100 - 300	Lindahl & Nyberg 1974
Base damages#	10,000	Richter et al. 1988
Single-strand breaks	20000-40000	Saul & Ames 1985
Interstrand cross-links	8	
Double-strand breaks	9	Bernstein & Bernstein 1991
DNA-protein cross-links	Unknown	

* It should be noted that the rates are calculated on the basis of spontaneous (endogenous) damaging events and therefore could be actually much higher depending on the dietary composition and style of living.

(Including all types of base damage Viz. oxidative damage, adduct formation with reducing sugars, methylation, cross links, and so forth)

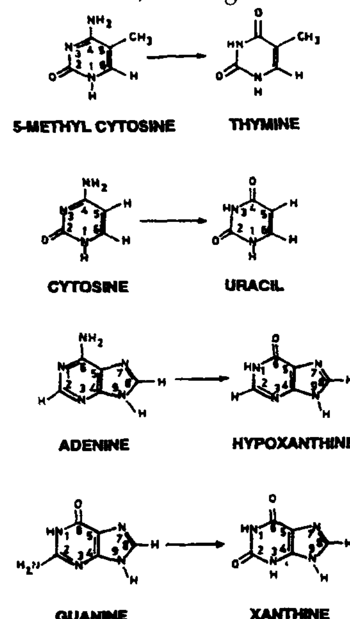

Figure 2 Some of the deamination products of DNA bases which could result in mismatches and eventually in altered base pairs.

Table 2 *Estimation of Single (SSB) and Double (DSB) strand breaks in permeabilised neurons of different ages by nick translation and terminal transferase assays.*

	Control	MNNG (50mM)	Glutamate (1mM)
Single strand breaks			
Young	3.0	5.4	14.0
Adult	4.6	15.0	24.0
Old	7.4	19.0	25.0
Double strand breaks			
Young	156	360	1080
Adult	276	960	1680
Old	600	2700	2280

Values of SSB are per neuronal cell ($x10^3$), while in the case of DSB they are the actual number per neuronal cell. Viability of the cells was judged by the trypan blue exclusion test. Young animals were 4 days old, adult were 6 months and old were >2 years. For other details see reference, Mandavilli and Rao 1996.

in the genomic DNA of an old neuron as compared with 3000 in young neuron. When the cells are exposed to either MNNG (a methylating agent) or glutamate (excitotoxic at higher levels) before assessment of the breaks, the damage is clearly aggravated at all the three ages studied, once again greatest damage is seen in old neurons indicating the increased susceptibility of genomic DNA with age. Even with respect to the DSBs, a steady increase in number is seen with age in neurons from cerebral cortex. There is a four-fold increase in the number between young and old ages. Prior treatment of cells with either MNNG or glutamate resulted in the formation of more of DSBs at all ages but the age dependent susceptibility of neurons to these genotoxic substances could be clearly seen.

The above studies of assessing SSBs and DSBs in brain cells with age have also been extended to examine the damage in neurons and glial cells isolated from different regions of the brain. The number of SSBs increases with age in both the cell types and in all the regions studied viz., cerebral cortex, cerebellum, hippocampus, hypothalamus and brain stem (figure 3). Highest number of SSBs was seen in neurons and astrocytes of cerebral cortex of any age. This also meant that cerebral cortex is the most vulnerable region for suffering DNA damage of this kind (Mandavilli & Rao 1996a). The next position of vulnerability goes to hippocampus in both types of cells. It is interesting that these two regions are known to be concerned with higher functions

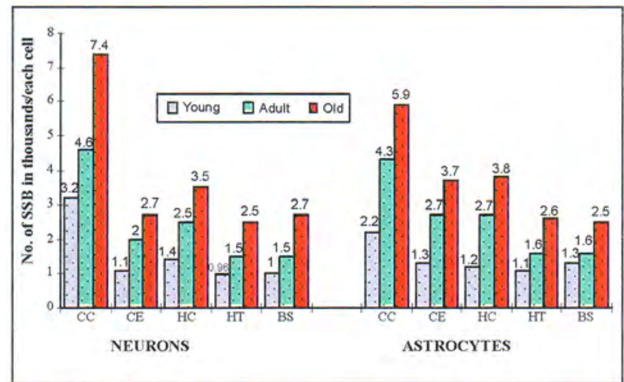

Figure 3 Single Strand Breaks (SSBs) in genomic DNA in neurons and astrocytes isolated from different regions of young, adult and old rat brains. Values are per cell (neuron or astrocyte) and in thousands. *CC*,cerebral cortex; *CE*, cerebellum; *HC*,hippocampus; *HT*,hypothalamus and *BS*, brain stem. For methodological details please see Mandavilli and Rao 1996a

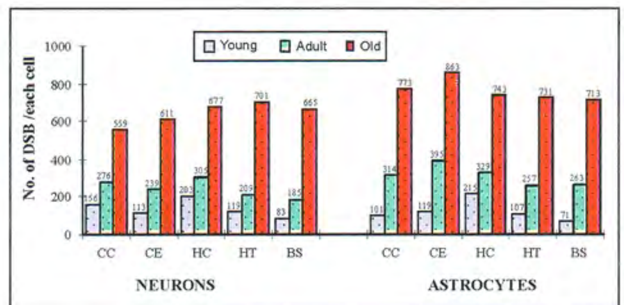

Figure 4 Double Strand Breaks (DSBs) in genomic DNA of neurons and astrocytes isolated from different regions of young, adult and old rat brains. Values are per cell, neuron or astrocyte. For methodological details please *see* Mandavilli and Rao 1996a.

like cognition and memory. In figure 4, the number of DSBs found in the cells of these regions was presented. The general trend of changes appears to be the same as in the case of SSBs. There is an increase in the number with age and glial cells harbor more DSBs with advancement of age in all the regions studied. However, all the regions are affected to the same extent because of old age.

Many other laboratories have also been looking at the changes in the DNA-damage profiles with respect to age and the tissue. In general the out come of all these studies is the conclusion that there is an accumulation of DNA damage with age in various organs including brain (Hosokawa et al. 2000, Hamilton et al. 2001, Schmerold & Niedermuller 2001). Mitochondrial DNA damage has been studied separately by some researchers in view of the gaining importance of oxidative damage to DNA as a more

specific pointer of aging (Bohr et al. 1998, Van Remmen & Richardson 2001, Wolf et al. 2002). For example, Chen et al. (2002) have most recently shown that there is an age-dependent decline of DNA-repair activity for oxidative lesions in rat brain mitochondria.

DNA-Repair Pathways

Just as the very metabolic activity in organisms is able to produce various types of DNA damage discussed above, many pathways to counteract such damage have also been generated by the same organisms. Basically, the DNA repair mechanism appears to be highly conserved in certain respects while markedly evolved in certain other respects. The last 30 years have seen great strides of advancement in the understanding of the various pathways of DNA-repair both in prokaryotes and higher organisms including humans. Excellent reviews have appeared in recent years about the existing knowledge of DNA-repair pathways in eukaryotes (Wood 1996, Hoeijmakers 2001). In mammalian cells, there are at least four major DNA-repair pathways. (1) A simple reversal of the damage; (2) Nucleotide Excision Repair (NER); (3) Base Excision Repair (BER); and (4) Recombinatorial repair including the end joining. In view of the limited scope of this article, only the BER pathway, in view of its relevance to brain tissue, will be discussed here and for details about other pathways the above mentioned review articles may be consulted.

Excision repair pathway is the most predominant and perhaps universal one to maintain the genomic integrity. This pathway includes mismatch repair as well and responsible for repairing a variety of lesions ranging from a simple methylated base to the inter strand adduct formation leading to a major distortion in the DNA structure. Essentially, the overall strategy in this pathway consists of 4 steps. (1) Recognition of the damaged site and making an incision at the locus; (2) Excision of the damaged portion; (3) Resynthesis of the removed sequence of the bases using the other strand of the DNA as template; and (4) Ligation of the newly synthesized strand with already existing downstream base.

As the knowledge about this DNA repair pathway began to increase it became clear that this pathway can be very easily viewed as two different pathways designated as Nucleotide Excision Repair (NER) and the Base Excision Repair (BER). An outline of these two pathways is shown in figure 5. NER seems to come into operation to repair such a DNA damage, which would cause a distinct helical distortion in DNA. Also excision patch is quite long and therefore there is need to resynthesise a long patch. For example, in the case of uv-induced damage the incision occurs precisely at 6 bases 3′ to the damage and 22 bases 5′ to the damage, thus releasing a 29 nucleotide fragment (Tanaka & Wood 1994). In mammalian cells the recognition and incision/excision processes require the participation of many proteins and the resynthesis of the patch is performed by a PCNA dependent polymerase (δ/ε) activity. For a detailed account, a review by Thompson (1998) is recommended. BER, on the other hand, comes into action when the damage to DNA is inflicted by the internal cellular events causing AP sites or modification of the bases including oxidation. Implicit in this mode of repair is the fact that the length of the excised portion and therefore that of the resynthesised patch will be

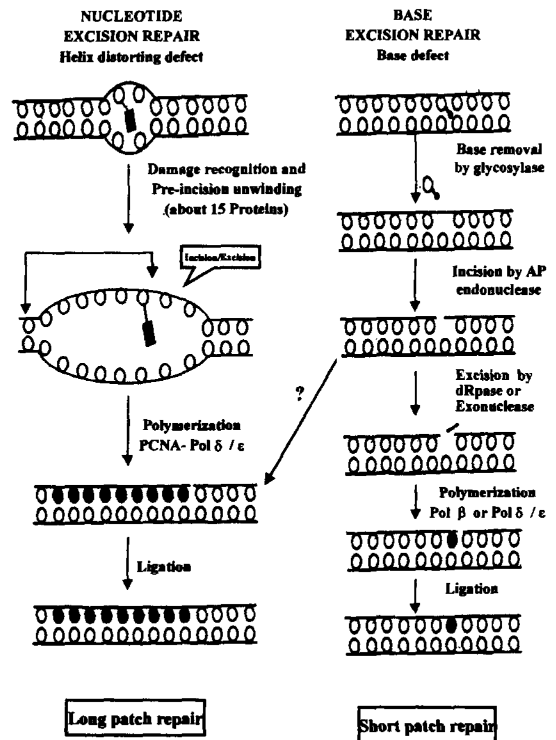

Figure 5 Pathways for DNA-Excision Repair. On the left and right are the Nucleotide Excision Repair (NER) and Base Excision Repair (BER) pathways respectively. The diagram is designed on the basis of presently available information and represents only the general outline but not the details.

shorter. It is for this reason, the NER (the left panel of figure 5) is considered as 'long patch repair' while the BER (right panel of the figure 5) is routinely considered as 'short patch repair'. However, it has now become clear that BER itself can be viewed as having two distinct sub pathways- the short and long patch pathways as discussed below.

Base Excision Repair Pathway

BER pathway consists essentially of 4 steps and can be divided into two sub pathways one concerned with 'short patch or single nucleotide replacing pathway' and the other 'long patch pathway' involving the insertion of up to 13 nucleotides (figure 6). In step one of short patch pathway (left panel of figure 6) the altered base (A) is recognized and cleaved from the deoxyribose phosphate moiety by an appropriate DNA-glycosylase, which also allows the AP endonuclease (APE1) to reach the site. (figure 6.1) As on today there are at least 8 glycosylases identified in human tissues with varying and overlapping specificities and this aspect has been reviewed recently by Lindahl and Wood (1999) and by Scharer and Jiricny (2001). Some DNA-glycosylases recognizing and removing 8-oxy guanine opposite C (Rosenquist et al. 1997, Radicella et al. 1997), oxidative forms of bases like thymine glycol, cytosine glycol, dihyrouracil (Hilbert et al. 1997) and alkylated adenine like 3-methyl adenine, ethenoadenine and hypoxanthine (Chakravarthi et al. 1991,Samson et al. 1991) are also known. It is likely that other types of DNA-glycosylases are yet to be discovered in view of the importance of this initial step in recognizing a whole array of possible damages that could occur to bases in DNA.

The second step (figure 6.2) consists of the breaking the chain at 5'-side of the abasic site. The major endonuclease specific for abasic site in DNA in humans is APE1, also known as HAP1, APEX, REF1 (Demple et al. 1991, Seki et al. 1992, Robson et al. 1992) Structurally, APE1 belongs to a super family of nucleases (Gorman et al. 1997). However, structural uniqueness at the active site of the enzyme consisting of extra loops seems to confer the specificity towards a baseless deoxyribose. The enzyme flips out the baseless deoxyribose and cleaves it on the 5' side. Also, like in the case of 1st step, this enzyme, still bound to DNA, attracts and interacts with pol b, which is involved in the next step in the repair pathway. The glycosylase dissociates from DNA at this point.

In step 3, the pol b fills up the one nucleotide gap and also releases the 5'-deoxyribose phosphate (dRp). At the same time DNA-ligase III-XRCC1 (X-ray repair cross complementing, gene1) complex arrives at the site.

Pol b is one of the five major DNA-polymerases found in mammalian cells and smallest in size with a single polypeptide chain of 335 amino acids with a molecular weight of 39 kDa. Both the rat and human

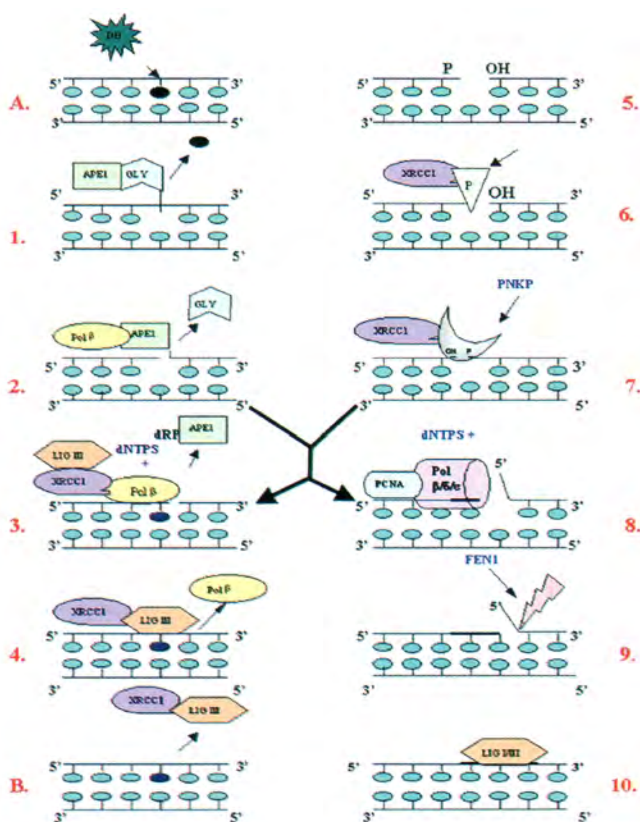

Figure 6 Sub-pathways of Base Excision Repair (BER): On the left and right side are the 'Short patch' or single nucleotide pathway and the 'long patch' pathways respectively. Crossing over of the pathways can occur at points 2 and 7. For detailed discussion of each step, please see text. Abbreviations: DB-damaged base; APE1-human apurinic/ apyrimidinic endonuclease 1; GLY-DNA-Glycosylase; Pol b/ d/ e- DNA-Polymerase b/ d/ e respectively; dRP-deoxyribose-5'-phosphate; XRCC1-X-ray repair cross-complementing, gene1; LIGI/III- DNA-ligase I / III; PARP1-Poly (ADP-ribose) Polymerase1; PNKP- Polynucleotide Kinase 3-prime phosphatases; FEN1- 'Flap' structure specific endonuclease 1; dNTPs- deoxynucleoside triphosphates. There is some recent suggestion that one of the several DNA-polymerases being discovered newly, the DNA-polymerase lambda (l) has similar properties as that of pol b and may participate in BER. More information in these lines is awaited.

enzymes were cloned 15 years ago and extensively studied over the years by Wilson and his group (SenGupta et al. 1986, Zmudzka et al. 1986) and by Matsukage' group (Yamaguchi et al. 1987, Date et al. 1988) The structural, catalytic and physiological aspects of pol β have been the subjects of two recent elegant reviews by Wilson and his associates (Wilson 1998, Idriss et al. 2002). Pol β is a single elongated polypeptide with two globular regions. These two regions can be separated by milder proteolysis, one N-terminal peptide with a molecular weight of 8 kDa and the other portion with a 31-kDa molecular weight. The larger fragment binds to the double stranded region at the 3'-OH end to be extended and carries out the polymerase catalytic function while the 8 kDa smaller fragment carries the deoxyribose phosphate lyase (dRpase) activity as well as single strand DNA binding activity. For further details about the biochemistry of pol β, the above-mentioned review articles may be referred to.

Step 4 consists of DNA-ligase III sealing the nick and pol β dissociating from the site. Subsequently the XRCC1 and ligase III come off from the site leaving behind repaired DNA (figure 6 left panel, B). Human XRCC1 has been cloned and is a 69.5 kDa protein with no observed enzyme activity (Thompson et al. 1990) It not only complexes with DNA-ligase III but also interacts with other core enzymes involved in BER and is therefore considered to play a crucial role in protein exchanges in the pathway (reviewed by Thompson West 2000). Eukaryotes, in contrast to prokaryotes, contain more than one DNA ligase, and these enzymes have distinct roles in DNA metabolism. Five DNA ligase activities, I-V, have been purified from mammalian cell extracts. Ligase III is more closely involved in DNA repair and recombination. Two biochemically distinct isoforms of ligase III, alpha and beta, encoding polypeptides with different C-terminal amino acids have been identified. The ligase III alpha isoform, which is most ubiquitously expressed and consists of 922 amino acids, interacts with DNA-repair protein, XRCC1 and affects the last step of nick sealing in BER pathway (Chen et al. 1995, Tomkinson & Mackey 1998, Wood et al. 2001)

The overall process is characterized by the sequential binding of proteins to DNA as well as among themselves in pairs facilitating the repair operation to occur efficiently and swiftly (Kohler et al. 1999, Hoeijmakers 2001).

The predominant route for BER is the 'short patch or single nucleotide pathway' discussed until now and shown on the left side of figure 6. In cases where the terminal sugar phosphate after the AP endonuclease incision (Step 2) develops a complex structure that cannot be acted upon by the dRpase activity of pol β (for example, reduced or oxidized abasic site) the repair synthesis would nevertheless continue but in a strand displacement manner (figure 6 right side panel) This long patch synthesis is catalyzed either by pol β itself or a bigger polymerase like pol δ/ε with associated proof reading activity. Also, this pathway is stimulated by Proliferating Cell Nuclear Antigen (PCNA) and requires a 'flap' structure specific endonuclease-1 (FEN1) activity to cut the flap like structure produced by the strand displacement type synthesis by pol β (Wu et al. 1996, Klungland & Lindahl 1997). The role of PCNA seems to be stimulation of FEN1 activity and the repair patch size is about 7 nucleotides (Frosina et al. 1996). It is now found that even pol β has a motif that would interact with PCNA and the significance of this is yet to be deciphered (Kedar et al. 2002).

There seem to exist a slightly different long patch BER pathway in which pol δ/ε(DNA-polymerase δ/ε) are involved instead of pol β. This was borne out by the observations that pol β null embryonic fibroblast cells were proficient in repairing oxidative damage although defective in uracil initiated repair (Sobol et al. 1996) and that a neutralizing antibody to Pol β, which inhibited repair synthesis catalyzed by pure Pol β by approximately 90%, only suppressed repair in crude human cell extracts by a maximum of approximately 70%. An inhibitor of Pol beta, ddCTP, decreased base excision repair in crude extracts by approximately 50%, whereas the Pol α/δ/ε inhibitor, aphidicolin, reduced the reaction by approximately 20%. A combination of these chemical inhibitors almost completely abolished repair synthesis. These data suggest that while Pol β is the major base excision repair polymerase in human cells but other polymerases also contribute to a significant extent (Nealon et al. 1996). Using pol β

deleted mouse fibroblast extracts and circular DNA containing a single AP site, it is shown by Stucki et al (1998) that the pol δ/ε participating 'long patch' BER requires PCNA and replication factor-C (RF-C). Subsequently Matsumoto et al. (1999) have reconstituted PCNA dependent repair of AP site with six purified human proteins: AP endonuclease, RF-C, PCNA, flap endonuclease 1, DNA Polymerase δ and DNA ligase I. The length of the patch size ranged from 2 to 7 nucleotides. The role of PCNA is envisaged to be acting like a scaffold for pol δ much the same way as XRCC1 for pol β in the short patch pathway.

From all the literature cited above, it does appear that the long patch repair can be affected both by pol β and pol δ/ε and characterized by the requirement of PCNA and FEN1 endonuclease. The ligation can be achieved, it appears, either by DNA-ligase I or III. It is now a matter of speculation as to what determines the type of DNA polymerase to be recruited for long patch BER pathway.

The involvement of three other proteins in BER is reported in literature. These are poly (ADP-ribose) polymerase-1 (PARP-1), polynucleotide kinase 3'-phosphatase (PNKP, also known as PNK, or DNA-kinase) and DNA-polymerase lambda (pol λ). These enzymes are envisaged to help the long patch repair process, entry of damaged substrates into BER pathway and providing a substitute for pol β activity respectively (Prasad et al. 2001, Jiliani et al. 1999, Garcia-Diaz et al. 2001). All these aspects are depicted in figure 6 and table 3 summarizes the factors involved in short and long patch BER.

Table 3 *Summary of various enzymes and other components involved in BER in Mammalian cells starting from a damaged DNA with an abasic site or a single strand break.*

Short Patch BER	Long Patch BER
(1nucleotide gap)	(2 to 13-nucleotide gap)
AP endonuclease (14q12)	AP endonuclease
Pol β with both dRPase and Polymerase activities (8p11.2) Pol Lambda (λ) with dRPase activity?	Pol β/pol δ/ ε
XRCC1 (19q13.2-q13.3)	XRCC1
PARP-1 (1q42)	PARP-1
PNKP (19q13.3-q13.4)	PNKP
DNA ligase III (17q11.2-q12) DNA ligase I (19q13.2-q13.3)	DNA ligase III DNA ligase I FEN1 (11q12) PCNA (20p12)

Some biochemical properties and supplementary genetic information provided by Wood et al (2001) can be viewed at the web site www.cgal.icnet.uk/DNA_repair_Genes.html. However, the chromosomal location of the concerned gene is indicated in the parenthesis. It is to be noted that what determines the entry of DNA with an basic site or single strand break into either short patch or long patch pathway is not completely yet clear. Similarly, once the long patch mode of repair is initiated to what extent pol β or pol δ/ε are involved in the filling up of the patch and the factors influencing this process thereof, is also uncertain at this point of time.

Brain Aging, BER and pol β

As already emphasized, in a non-replicating tissue like brain endowed with high metabolic activity, BER would be the main guardian to ensure genomic stability. However, much of the information regarding the details of BER pathway has come from studies on tissues other than brain although it is generally supposed that the pathway must be essentially the same. With the advent of several neurodegenerative diseases making their appearance in old age and the accumulating knowledge of their molecular link to genomic stability (Martin 1999, Sniden 2001). the status of BER in health and disease assumed great importance. Indeed several studies in recent times were directed towards assessing either the overall pathway of BER or a particular step in that pathway in brain. Quite often these studies are directed towards understanding the DNA repair status in brain under a given experimental condition rather than establishing the existence of BER pathway in nervous tissue. Nevertheless, these studies have also served the purpose of providing evidence that indeed the BER pathway does exist in brain. Table 4 summarizes the information available at this time regarding the existence various enzymes and other accessory factors known or suspected to be participating in BER, in brain.

The relationship between DNA repair and the phenomenon of brain aging has been the subject of study in this laboratory for the past several years. Since pol β is an important component of BER machinery, a systematic study to assess the levels of this enzyme in young, adult and old rat brain was undertaken through, western and northern blotting. Activity gel assays coupled with immunotitration experiments were also performed

Table 4 *Base Excision Repair (BER) Machinery in Brain*

Enzyme	Mechanism of Action	Reference
Uracil-N-Glycosylase	Cleavage of glycosidic bond releasing uracil	Krokan et al. 1983 Weng & Sirover 1993
AP-endodeoxy nuclease	Incises near AP sites in DNA on the 5′-side	Ivanov et al. 1988 Wilson et al. 1996 Lewen et al. 2001
XRCC1	Interacts with Pol β, DNA Ligase III and PARP and participates in BER	Fujimura et al. 2000
Poly (ADPR) Synthase/polymerase	Protects strand interruptions in DNA. Activated by single strand breaks in DNA. Catalyses the poly ADP-ribosylaton of nuclear proteins. Activates DNA-repair (BER). Acts as a source of ATP for ligation step.	Bilen et al. 1981 Strosznajder et al. 2000
PCNA	Promotes DNA Polymerase δ/ε activities. Participates in long patch BER pathway. Interacts with pol β and FEN1.	Ino & Chiba 2000
FEN1	Flap structure specific endonuclease 1 that cleaves 5'flap structure and fails to cleave other DNA structures, including 3'flaps and single stranded DNA (by similarity). Involved in long patch base excision repair where it interacts with PCNA complex and DNA and acts as an exonuclease.	Otto et al. 2001
Pol β	The smallest of the DNA polymerases found in mammalian tissues including brain. Considered essentially as a repair polymerase. Largely responsible for BER of DNA damage. Conducts template dependent short "gap-filling" DNA synthesis. Most predominant DNA polymerase in brain	Waser et al. 1979 Rao et al. 1985 Rao et al. 1994 Prapurna & Rao 1997
Poll δ/ε	Involved in both DNA replication and repair including long patch BER.	Prapurna & Rao 1997
DNA-Ligases-I &III	DNA joining, require ATP. Ligase I interacts with pol β. Ligase III interacts with XRCC1	Inoue & Kato1980 Tomkinson & Mackey 1998
*DNA-methyl transferase	Methylation of bases in DNA possibly for restoration of original methylation status after mismatch repair	Brooks et al. 1996
*Topoisomerase II β	Relaxation, super coiling of DNA	Watanabe et al. 1994
"3′-5′ exonuclease ′	Excision of bases from 3′-end May help pol β in proof reading during BER	Rao et al. 2000 and Unpublished observations in author's lab
*Acid and Alkaline DNases from Chick and Rat Brain	Acid DNase attacks native DNA and UV irradiated DNA	Suvarchala & Rao 1994
	Alkaline DNase degrading DNA with no clear specificity but showing higher activity towards damaged DNA. Both the enzymes are endo nucleases	Venugopal & Rao 1993

* The role of these enzymes in BER is not known at this time. However, mention is made of these activities found in brain since their precise role in DNA metabolism in neurons is likely to be known in the years to come.

in order to reach meaningful conclusions as far as the activity levels of this polymerase are concerned vis-à-vis the age of the animal (Rao et al. 1994). The results of all these experiments are summarized in figure 7. As can be seen, by western blotting a reduction of 30% and 20% in the pol b levels was seen in adult (6 months) and old (more than 2 years) brains respectively, as compared to the values in young (4 days post natal) brains. Similarly, Northern analysis has shown a reduction of pol b mRNA by 21% and 29% in adult and old brains respectively. However, when the actual enzymatic activity was measured by activity gel assay (measuring the activity on the polyacrylamide gel itself after separating the proteins of the brain extract by electrophoresis and incubating the whole gel under pol b assay conditions), the decrease in the activity was more than that found either with western or northern. Thus, in adult brain the activity went down by 45% while in old brain the fall was 53% in comparison with the young activity. These results brought up the possibility that some of the pol b molecules synthesized are not catalytically active but immunologically competent. The immunotitration experiments showed that indeed this seems to be the case. When equal amount of pol b activity from young, adult and old brain extracts were taken and titrated with increasing amounts of polyclonal antibody to pol b, it was seen that more of antiserum is needed to reduce the activity by 50% in old extracts as compared to the amounts of antiserum required to bring about the same reduction in activity with young and adult extracts. The antiserum required to affect 50% reduction in the pol b activity in young, adult and old extracts is 12, 22, and 34 ml respectively. These studies thus demonstrated that with the advancement of age, not only the levels of pol b go down in brain but there is also the accumulation of catalytically incompetent pol b molecules. It is at the speculative level whether this is due to post-translational modification of the enzyme molecules. Be that as it may, it is certain that the pol b activity in brain decreases in an age dependent manner, which is bound to affect adversely the DNA repair potential of this organ. In order to substantiate this aspect, pol b activity in isolated neuronal and astroglial cell fractions from rat brain cortex of three different ages mentioned above was measured and the data are shown in table 5. Calf thymus 'activated' DNA was used as template primer in these experiments. It is once again clear that even in isolated cells the reduction in this polymearse activity occurs in a telling manner more so in the neuronal cells. In glial cells the decrease with age was there and statistically significant but the animal-to-animal variation was more for these cells (Raji et al. 2002).

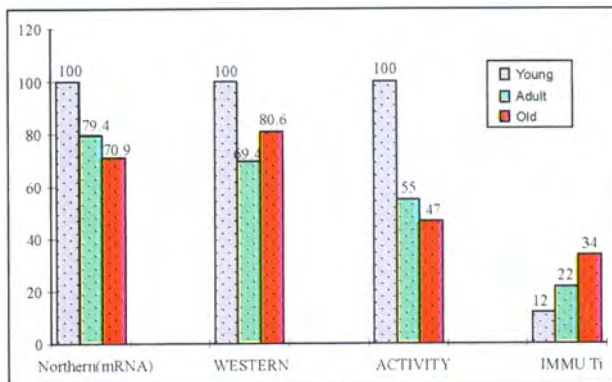

Figure 7 Assessment of 'active' levels of pol b in aging rat brain through different strategies like Northern blotting, Western blotting, activity gel assay and immunotitration. In the case of northern, western and activity gel, equal amounts of RNA/protein was loaded and the values represent relative percentages with that of 'young' taken as 100. In the immunotitration experiments, equal amount of activity was taken in all cases and the values denote the microlitres of pol b antiserum required for inhibiting the activity by 50%. For other details please see text and also Rao et al. 1994.

There was however, a need to show that pol b dependent DNA–repair is indeed adversely affected in brain cells with age and for this a more physiologically relevant assay for pol b activity was required. A deoxyoligo duplex with staggered

Table 5 *DNA Polymerase activity in rat neuronal and astroglial cells of different ages*

Substrate and Cell Fraction used	Age		
	Young	Adult	Old
'Activated DNA'			
Neurons	2023±1076	719±541*	568±412*
Astroglia	1471±550	822±512*	694±652*

Values are averages ± S.D. and expressed as Pico moles of the radioactive deoxy nucleotide incorporated into the acid insoluble fraction in 1 hr / mg protein. Eleven and nine independent experiments were performed in the case of neurons and astroglia respectively. While there was considerable variation in the activities from one animal to the other, the trend of changes with age and a given substrate were quite similar.

* These values are significantly different (p< 0.001 for neurons and 0.02 for Astroglia respectively) from the corresponding value at 'Young'. Data taken from Raji et al. 2002.

ends was used as a model substrate to examine the ability of neuronal extracts from rat brain of different ages, to extend the primer (14-mer) to the template length (21-mer).

The sequences of the synthetic deoxyoligonucleotides used in this study are shown below. When each of the 21-mers is hybridized with the 14-mer primer, which is pre-labeled on 5' side with ^{32}P, one of the resultant oligoduplex has the correct complimentary base (Cytosine) at 14th position from 3'-end (that is opposite to the 3'-end of the 14-mer primer) whereas the other three have at that position the three mismatched bases (T or, G or, A) as follows.

(1) 5'-cgcgatcggtagc**G**-3' (oligo 1 - 14mer)

(2) 3'-gcgctagccatcg**C**gttaccg-5' (oligo 2 - 21mer-C)

(3) 3'-gcgctagccatcg**T**gttaccg-5' (oligo 3 - 21mer-T)

(4) 3'-gcgctagccatcg**G**gttaccg-5' (oligo 4 - 21mer-G)

(5) 3'-gcgctagccatcg**A**gttaccg-5' (oligo 5 - 21mer-A)

5'-cgcgatcggtagc**G**-3'
3'-gcgctagccatcg**C**gttaccg-5' | Correctly matched duplex

5'-cgcgatcggtagc**G**-3'
3'-gcgctagccatcg**T**gttaccg-5'
5'-cgcgatcggtagc**G**-3'
3'-gcgctagccatcg**G**gttaccg-5' | Mismatched duplexes
5'-cgcgatcggtagc**G**-3'
3'-gcgctagccatcg**A**gttaccg-5'

Then, the four oligoduplexes were presented to neuronal extracts prepared from young, adult and old rat brains under appropriate DNA polymerase assay conditions. If the extracts have adequate amounts of polymerase, then the 14 mer would be extended to the template length (21 mer) through the addition of 7 nucleotides. The extension activity was measured by subjecting the products to sequencing polyacrylamide gel electrophoresis. It was found the while the neuronal extracts of all ages were able to degrade the primer to shorter lengths; only feeble extension of the primer to the longer lengths was seen even with 'young' extracts. In the case of 'adult' and 'old' extracts extension was almost undetectable. Within this limited activity, the correctly matched primer was extended to the maximum extent while all the mismatched primers showed very little extension indicating that extension of primer with a mismatched base pair is not preferred (figure 8). After many trials, it was possible to establish conditions to restore this lost

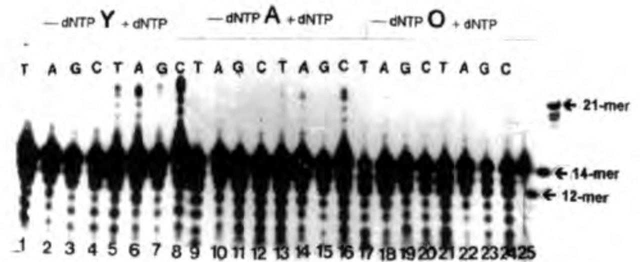

Figure 8 Exo-extension activities of rat brain neuronal extracts with correctly matched and mismatched oligo duplex template primers. Details regarding the incubation conditions and identification of the products through sequencing gel electrophoresis can be seen from reference Rao et al, 2000 from where the figure is taken. The reaction was carried out both in the presence (20 mM) and absence of dNTPs. Lanes 1-8 contain neuronal extracts from young brain (Y- 5 days postnatal), lanes 9-16 contain neuronal extracts from adult brain (A- 6 months and 21 days old) and lanes 17–24 contain neuronal extracts from old brain (O-28 months old). At each age, the first four tubes are without dNTPs while the next four are with dNTPs. Lane 25 is without any neuronal extract (enzyme blank). The mobilities of standard 21-, 14- and 12-mer are shown. The base in 21-mer corresponding to that at the 3'-end of the primer is shown on the top. In all the tubes protein in neuronal extracts was normalized to a fixed amount by adjusting the volume (usually 5 or 10 mg). Similarly equal amounts of products were loaded for the sequencing gel electrophoresis.

primer extension activity even in neuronal extracts from "old" brain (Rao et al. 2000). The strategy consisted of supplementing the neuronal extracts with pure rat liver pol b after the mismatched base at the 3'end of the primer is removed. Among the various polymerases tested, pol b gave most satisfactory results. The restored primer extension activity in a typical experiment is shown in figure 9. It is imperative that pol b activity is to be assessed with other model substrates before any definitive conclusions can be reached about the restorability of BER activity in aging neurons. Nevertheless, the above observations suggest some probability for achieving that.

During the past two years, a number of reports appeared which essentially substantiate the above observations. Thus, LeDoux and Wilson (2001) reviewed the BER of mitochondrial DNA-damage in mammalian cells and discussed the biological consequences of inadequate BER. An age dependent reduction in BER activity for oxidative lesions in DNA of rat brain mitochondria was demonstrated by Chen et al. (2002). Studies with male germ cell nuclear extracts prepared from

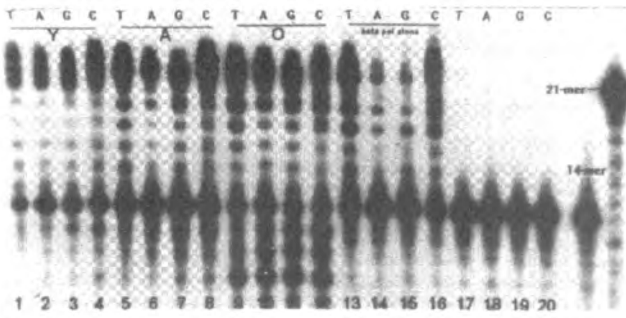

Figure 9 Two-step reaction to assess the effect of prior incubation of the oligo duplex template primers with the neuronal extracts (young-Y, adult-A and old-O) on the primer elongation achieved by b-pol in the subsequent step. During the first step, the four oligo duplexes were incubated with neuronal extracts for 10 min at 37° C in the absence of any exogenous polymerase and dNTPs. The reaction was stopped by heating at 70° C for 10 min. In the second step 20mM dNTPs and b-pol (2.5 units) were added and the incubation at 37° C was continued for 20 min. in the presence of manganese replacing the magnesium in the buffer. Figure taken from Rao et al. 2000.

young and old mice have shown that the BER activity is limited by the reduced levels of AP endonuclease in old animals (Intano et al. 2002). Increased methyl methanesulfonate induced mutation frequency was seen in pol b null mouse embryonic fibroblasts (Sobol et al. 2002). BER pathway was studied in different mouse tissues (brain, liver, spleen and testis) at young (4 months) and old (24 months) ages by Cabelof et al. (2002). In all the tissues tested, an age dependent decrease in the repair capacity was observed and this was found to be due to reduced levels of pol b activity, protein and mRNA. Further, these workers have also shown that both spontaneous and methyl methanesulfonate induced mutation frequency was very high in old animals. All these above findings underline the importance of BER pathway and it's key component, pol b in maintaining the genomic integrity. In such tissues like brain where BER constitutes the main DNA-repair pathway, the importance of this mode of repair can hardly be exaggerated. This is indeed borne out by the fact that mice with a knock out of any one of the majority of the genes involved in DNA-repair do show pathological phenotypes like retarded growth and higher cancer incidence etc., but are still viable. However, knocking out of certain genes like pol b, AP endonuclease, XRCC1, XRCC4, DNA ligase-1, DNA ligase-4 shows severe consequences like embryonic lethality (Friedberg & Meira 2000). It is noteworthy that most of these genes are involved in BER. More importantly, pol b knocked out mice were found to suffer from abnormal neurogenesis, extensive apoptotic cell death of newly formed neurons and finally neonatal lethality (Sugo et al. 2000). These results, once again demonstrate the crucial role for BER and that of pol b in the maintenance of genomic integrity in brain.

Epilogue

With the advent of improved longevity of human population, a number of age dependent disabilities/ diseases are making their appearance-sometimes rendering the achievement of extended longevity meaningless. A number of such diseases are related to brain function and are collectively termed as 'neurodegenerative disorders'.

Efforts to increase the "health span" of aging population must therefore be directed towards at least reducing the severity of these age dependent neurological disorders. One way to achieve this might be to ensure sustained BER in brain cells since many of the neurological disorders are linked to mutation in one or more genes. Thus, major directions of future neurogerontological research might deal with more fundamental studies on BER in brain to know the most vulnerable component of BER pathway and more importantly the timing of the onset of this vulnerability in the life span. Secondly, efforts are to continue to translate or extend the observed *in vitro* benefits of restoring the BER to an *in vivo* situation. This will not be easy in spite of the upcoming stem cell technology since once differentiated the cells might lose the vigorous BER activity that was present in undifferentiated state. Hence the efforts may have to be channeled to make the terminally differentiated neurons competent with respect to BER pathway, for eventual use for therapeutic purposes.

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