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Genomic Damage and Its Repair in Young and Aging Brain

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Abstract

A brief review of the available information concerning age-related genomic (DNA) damage and its repair, with special reference to brain tissue, is presented. The usefulness of examining the validity of DNA-damage and repair hypothesis of aging in a postmitotic cell like neuron is emphasized. The limited number of reports that exist on brain seem to overwhelmingly support the accumulation of DNA damage with age. However, results regarding the age-dependent decline in DNA-repair capacity are conflicting and divided. The possible reasons for these discrepancies are discussed in light of the gathering evidence, including some human genetic disorders, to indicate how complex is the DNA-repair system in higher animals. It is suggested that assessment of repair potential of neurons with respect to a specific damage in a specific gene might yield more definitive answers about the DNA-repair process and its role in aging.

Index Entries: DNA damage; DNA-repair; brain; aging; genetic disorders.

Introduction

Ever since the structure of DNA was elucidated by Watson and Crick in 1953, it has been generally assumed that genomic DNA, in view of its vital role in transferring hereditary information from generation to generation, is a stable molecule unaltered in its structure by the surrounding events. This taken for granted, its remarkable attribute of stability has turned out to be a myth. As noted by Haynes (1988) DNA is made up of rather ordinary molecules that are not endowed with any peculiar kind of quantum mechanical stability. As such, DNA must be able to undergo all kinds of structural modifications at the body temperature and with many other chemicals in proximity. Much evidence has accumulated in recent years to prove that this is indeed the case, and normal cellular metabolism itself is enough to cause various types of damage to the genomic apparatus. If the genomic DNA can be assaulted in so many ways the natural question that would emerge is: How is genetic informational integrity maintained and transmitted through generations?

Extensive work during the past decade mainly using the prokaryotic systems has indicated that organisms also evolve efficient metabolic machinery to counteract or repair any DNA damage that might occur. There is an equilibrium between these two opposing events. When the equilibrium is in favor of DNA-repair, the genomic integrity is maintained; if it is the other way, then damage and mutations accrue that may lead to cancer and other age-associated diseases.

The brain is the master organ of the body. It controls all other functions either directly or indirectly. The brain has two major types of cells, the neurons and glial cells. It is known that neurons, once differentiated are nondividing, and even in glial cells only a small fraction of them are dividing in adult and old ages (Korr, 1980). Thus it can be considered that most of the cells in an adult brain are postmitotic. Further, in the majority of the species the final number of differentiated neurons is reached very early in life (Dobbing, 1971) and therefore a neuron's life-span is almost equal to that of the whole animal. Considering the high metabolic activity in a neuronal cell, it must be of great necessity and importance to maintain the genomic integrity over a long period of time in order to keep up the fidelity of the cellular processes. Thus the processes of genomic damage and its repair assume special significance in nervous tissue.

It is the purpose of this article to review briefly the manner in which genomic structure, that of DNA in particular, could be altered, and the metabolic machinery through which such damage could be reversed or repaired. Then a closer look will be taken at the information available about those events with reference to brain of different ages. Admittedly, the purpose of this article is not to point out how abundant the available information is but rather to expose how little is known about this vital process in a master organ like the brain endowed with the most advanced faculties, like learning, memory, and perception.

Genomic (DNA) Damage

It is quite evident now that all organisms, unicellular or multicellular, are constantly exposed to internal (spontaneous) and external (environmental) damaging factors (heat, free radicals, glucose, microorganisms, various kinds of radiation, and so forth) that are potentially dangerous for the maintenance of the structural as well as functional integrity of the genomic apparatus in the cell. Since DNA and protein are the two major constituents of the genome, the term genomic damage could be defined as any alteration or modification in the chemical structure, including the sequence changes, of macromolecular components of the genome and their proper interaction. As DNA is the material carrying the genetic information (except in the case of RNA viruses), the term genetic damage is routinely viewed as DNA damage and indeed the two terms are interchangeably used. However, it is equally important that the DNA must be in the proper

Table 1

Various Endogenous and Exogenous Factors/Sources of DNA Damage*

Endogenous (spontaneous) sources
Normal body temperature (37°C)
Normal cellular metabolism
Free radicals produced during metabolism
Errors in DNA replication and repair
High levels of reducing sugars
Methylating compounds like *S*-adenosyl methionine
Exogenous (environmental) sources
Dietary mutagenic compounds
UV and ionizing radiations like X-rays, γ-rays.
Free radicals (produced because of a variety of factors including ionizing radiations)
Heavy metals (Hartwig and Beyersmann,1989)
Ultrasound (Miller et al., 1989)

*Information obtained largely from Mullaart et al. (1990) and Bernstein and Bernstein (1991). When the source is different, it is indicated.

orientation in the overall structure of chromatin for maintaining functional efficiency. Therefore, any change in the overall chromatin architecture should also be considered as genetic damage.

A large body of information exists today to indicate how genomic DNA can be damaged or altered. All this information has been exhaustively reviewed by Friedberg (1985) and recently by Bernstein and Bernstein (1991). Table 1 summarizes various causative agents or factors that induce DNA damage in living organisms.

Broadly the causative factors can be divided into two main categories: the endogenous and the exogenous or environmental (Table 1). The endogenous sources include the normal metabolism itself, the body heat, free radicals generated during the metabolism, glucose and other reducing sugars, alkylation, and so forth. The effects of these factors on DNA are found to be mismatched base pairs, alteration in the structure of bases like tautomeric shifts and deamination, loss of bases (depurination and depyrimidination) resulting in apurinic/apyrimidinic sites (AP sites), single-strand and double-strand breaks (SSB and DSB). The exogenous or environmental factors that cause DNA damage include various types of radiation and dietary chemicals that can alkylate or act as free radical generating agents. These environmental factors can cause crosslinks, formation of bulky adducts, and oxidative cleavage in the DNA apart from the other types of changes listed above under endogenous factors. A look at the various types of damage or alterations that are seen in DNA, irrespective of the causative agent, seems to indicate that all these changes can be viewed basically as six different types (*see* Fig. 1)—AP sites, altered bases, thymidine dimers, crosslinks, SSB, and DSB—with the good possibility of one type getting converted to the other.

AP (Apurinic/Apyrimidinic) Sites

It is well established now that mere body temperature (37°C) is enough to cause the breakage of the *N*-glycosidic bond between the purine or pyrimidine base and the deoxyribose resulting in AP or baseless sites. The frequency of this breakage is higher at elevated temperatures. It is estimated by Lindahl and Nyberg (1972) and Lindahl (1977) that at the normal body temperature about 10,000 depurinations and 500 depyrimidinations occur/mammalian cell/d. Usually all these baseless sites are converted into SSB if not repaired. Saul and Ames (1985) deduced that about 20,000–40,000 such breaks take place/cell/d. Note that unrepaired AP sites

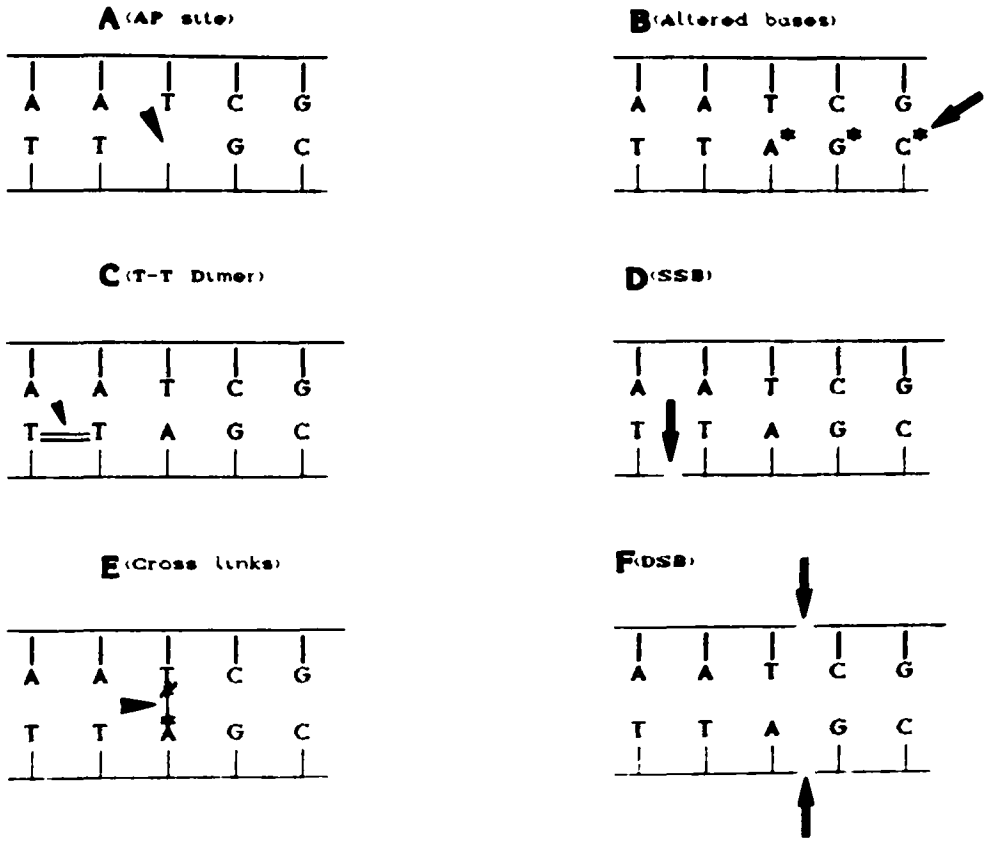

Fig. 1. Major modes of DNA damage. **A**, apurinic/apyrimidinic site (AP site). **B**, altered/modified bases that may result in mismatches. **C**, thymidine dimer, a major photoproduct formed owing to UV irradiation (250–260 nm). **D**, single-strand break, one of the most frequent consequential end points of various damages. **E**, crosslinks formed between two strands of DNA (interstrand). Sometimes the crosslinks are between DNA and the surrounding proteins. **F**, double-strand breaks—an occasional end point of oxidative/ionizing radiation induced damage.

in DNA have been shown to be mutagenic by causing GC→TA and AT→TA mutations (Schaaper and Loeb, 1981; Loeb and Preston, 1986). In a nonproliferating but long living cell like neuron of a human brain, about 10^8 purines are lost because of spontaneous depurination during a lifespan. This amounts to about 3% of the total number of purines in the cell (Lindahl and Nyberg, 1972).

Altered Bases Including Mismatches

At normal body temperature several reactions occur that modify the chemistry of bases. For example, deamination of cytosine to uracil, 5-methylcytosine to thymine, and, to a lesser extent, adenine and guanine to hypoxanthine and xanthine, respectively, are reported (Lindahl, 1979). Some of these products of deamination (Fig. 2) are potentially mutagenic since during the next round of semiconservative synthesis of DNA they are miscoding lesions that would result in altered base pairs. It is calculated that spontaneous chemical deamination of 5-methyl cytosine alone could create about 12 T:G mismatches/genome/d in mammalian cells (Brown and Jiricny, 1987). Although this type of DNA damage in a nonreplicating cell like neuron may not be of great relevance, it is still possible that such altered bases could interfere with the fidelity of transcriptional process.

Another spontaneous base modifying reaction is the methylation of DNA by *S*-adenosyl methionine (SAM) leading to the formation of N^7-methylguanine, N^3-methyladenine, and O^6-methylguanine (Barrows and Magee, 1982; Rydberg and Lindahl, 1982). These methylated bases can lead to baseless sites and eventually to strand breaks.

Cyclobutane Type Pyrimidine Dimers

Ultraviolet irradiation is one of the best studied DNA-damaging agents. Among other things, UV light of wavelengths around 260 nm induces the formation of chemical bonds between adjacent pyrimidines in DNA. The covalent linkage resulting from the saturation of 5,6 double bonds of pyrimidines is referred to as a cyclobutane dipyrimidine or pyrimidine dimer. The most readily formed dimer is that of the cyclobutane thymine dimer (Fig. 3). Although theoretically there can be 12 isomeric forms of the dimer, in UV exposed B-DNA the dimers are thought to exist exclusively in the *Cis-Syn* form (Kittler and Lober, 1977). Dimerized pyrimidines are extraordinarily stable to extreme pressures and temperatures. Thus these lesions survive total acid hydrolysis of DNA and can be easily separated from monomeric thymine. This would also mean that pyrimidine dimer can be a hindrance for the template activity of the DNA.

The other photoproducts in DNA subjected to UV irradiation are the noncyclobutane-type pyrimidine adducts, the typical examples being thymine glycol (Yamane et al., 1967), cytosine hydrate (Kittler and Lober, 1977), and pyrimidine-pyrimidine (6-4) lesions (Lippke et al., 1981). These structures are shown in Fig. 4. In addition, UV radiation also induces other kinds of DNA lesions including DNA-protein crosslinks and SSB (Hariharan and Cerutti, 1977; Peak et al., 1985; Lai et al., 1987). It is argued that this kind of lesion may be more relevant to a phenomenon like aging than the commonly studied pyrimidine dimers, since the wavelength of the majority of

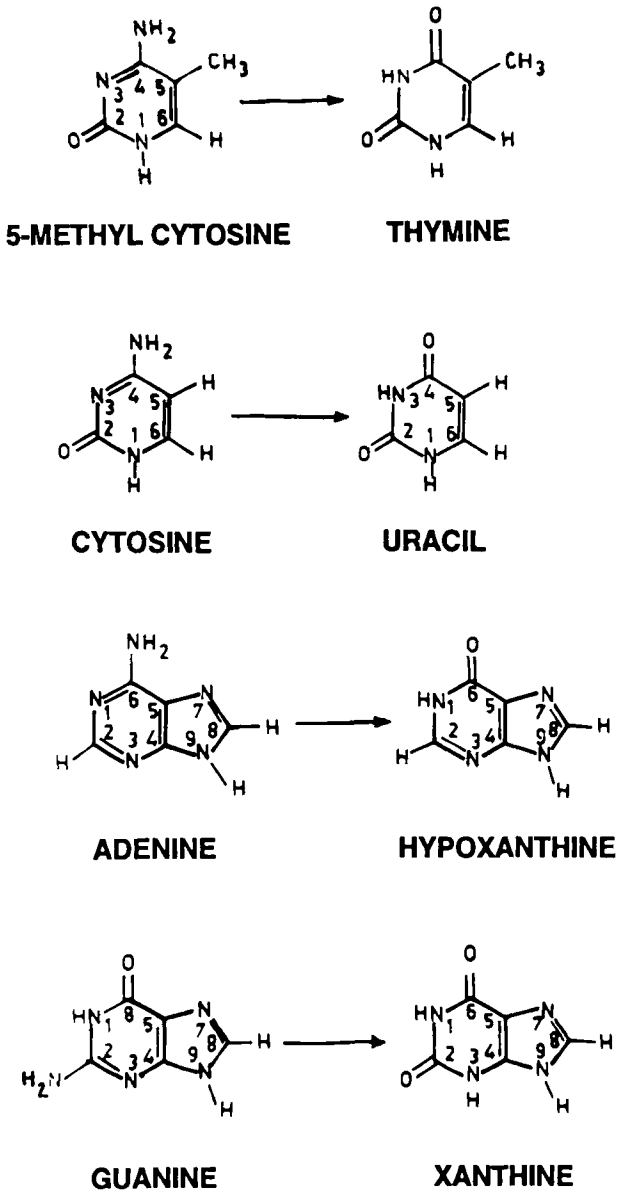

Fig. 2. Some of the deamination products of DNA bases.

the UV radiation that reaches earth's surface through sunlight is >320 nm. At this wavelength the predominant alterations induced in DNA are thought to be DNA-protein crosslinks and single strand breakage.

Any effect of UV light should be limited to skin in higher animals, since UV can hardly penetrate the skin and reach the tissues. In the brain,

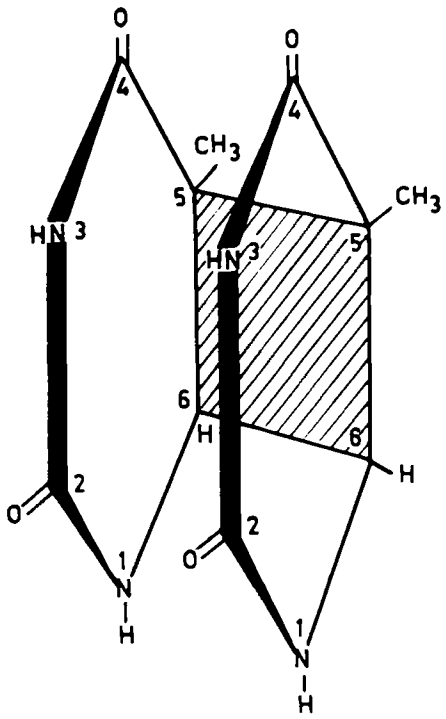

Fig. 3. The cyclobutyl thymine dimer, a major photoproduct of UV irradiation (254 nm) of DNA.

chances of UV-dependent damage to cellular DNA appear to be even more remote in view of the protection offered by the skull. However, it is interesting that there is a report to show that marsupial brain has photolyase activity—an enzyme that catalyzes the monomerization of pyrimidine dimers (Rupert, 1975). Further, the model system for the repair of UV-induced DNA damage is routinely used with various tissues including the brain, and it is abundantly clear now that all these tissues do carry out repair of UV-induced DNA-damage. It therefore appears that there might be UV mimetic events going on in tissues to damage the DNA and the relevant repair capacity has been evolved. Alternatively, the damage induced by UV light is common or similar to that brought about by some other events and a common repair pathway comes into operation.

Single-Strand Breaks (SSB)

Apparently, the most prevalent type of DNA-lesion in mammalian cells is the SSB. It appears that a single-strand breakage is the "end-point" of several other types of structural insults inflicted by a whole array of agents. As mentioned, an AP site may be converted to an SSB in alkaline pH. Similarly, bases that can be modified in the ways as discussed above may be removed by a suitable glycosylase, thus creating an AP site that would eventually result in an SSB. Also, the very DNA excision repair process itself may produce an SSB in an attempt to remove the damaged portion of the DNA strand (this aspect will be discussed later). In addition, UV light and ionizing radiations can cause SSB by direct or indirect action through generation of free radicals (for a review, *see* Mullaart et al., 1990). Human diet contains a great variety of natural mutagens and carcinogens such as polycyclic aromatic hydrocarbons, aflatoxins, and nitrosamines (for details, *see* Ames, 1983). These compounds can react with DNA, resulting in several types of damages including SSB, DSB, and bulky adducts. Bulky adducts of DNA have also been detected in tissues of heavy smokers (Randerath et al., 1986).

Double-Strand Breaks (DSB), Crosslinks, and Oxidative Damage

As explained above, UV, X-ray and γ-ray radiations can cause SSB, crosslinks, and DSB. Interstrand DNA-crosslinks represent an important class of chemical modification, since they prevent the DNA strand separation needed for replication and transcription processes. In fact, some of the compounds like mitomycin (Iyer and Szybalski, 1963) and photoactivated psoralens (Cole, 1970) are used in cancer chemotherapy precisely because of their property to create interstrand crosslinks in DNA and thus retard the replicative process.

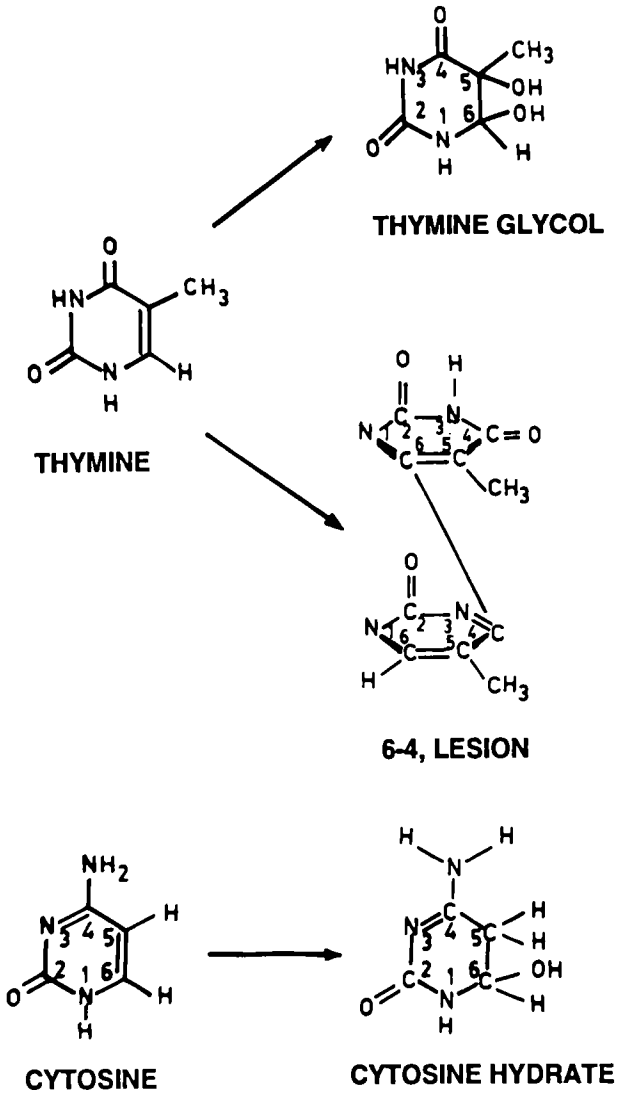

Fig. 4. Noncyclobutane-type damage to pyrimidine bases in DNA by UV irradiation. Product like thymine glycol is also known to result from oxygen radical induced damage.

The highly reactive superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) occur as byproducts of normal cellular respiration (Harman, 1981). These compounds can cause oxidative damage to DNA. There is evidence to show that repair of such oxidative damage leads to the removal of thymine glycol, thymidine glycol, and hydroxymethyl uracil, and these compounds are excreted in the urine (Saul et al., 1987). The levels of these compounds in urine are taken to reflect the rate of oxidative damage to DNA. It is also pointed out that these products are only three out of a considerable number of possible oxidative DNA damage products. Another important product of oxidative DNA damage, the 8-hydroxydeoxyguanosine (Kasai et al., 1986), was found to be present at a significant level in rat liver (Richter et al., 1988) and this oxidized base has been shown to be mutagenic (Shibutani et al., 1991). Lesko (1982) has presented evidence that H_2O_2 also produces DNA-protein crosslinks that will be an impediment for both replicative and transcriptional activities.

An estimate of the frequency of occurrence of DSB can be derived from the rates of occurrence of oxidatively damaged bases in vivo. The in vivo data can be obtained by extrapolating the in vitro observations. Modified base, DSB, and interstrand crosslinks are produced in vitro by H_2O_2 at a ratio of 120:1.1:1 (Massie et al., 1972). From this ratio, assuming that it is applicable to in vivo situations and taking that about 960 bases are oxidatively damaged/cell/d, the number of DSB and interstrand crosslinks in mammalian cells could be calculated as 8.8 and 8.0/cell/d, respectively (Bernstein and Bernstein, 1991). Double-strand damages can only be repaired by physical recombination with another homologous DNA molecule. Because of this, double-strand damages and in particular the DSB may play a critical role in survival of a nonreplicating cell like neuron.

Damage to Chromatin Structures

The discussion so far has largely been confined to the various types of damage to DNA and in particular to the bases. However, the functional efficiency of the DNA molecule would also depend on its proper conformation in the overall organization of chromatin. In eukaryotes, the higher level of compact organization of genomic

Table 2

Approximate Frequencies of Occurrence of DNA Damages in Mammalian Cells*

Type of damage	Events per cell per day	Reference
Depurination	10,000	Lindahl and Nyberg (1972)
Depyrimidination	500	Lindahl and Karlstrom (1973)
Deamination	100–300	Lindahl and Nyberg (1974)
Base damages (includes 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	Saul and Ames (1985)
Interstrand crosslinks	8	Bernstein and Bernstein (1991)
Double-strand breaks	9	
DNA-protein crosslinks	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.

DNA involves its association with both histones and nonhistone proteins. The association with histones results in the organization into repeating units called nucleosomes, which consist of a core of 140 base pairs (bp) of DNA wrapped around an octomer of histones that in turn is made up of two each of the histones H2A, H2B, H3, and H4 (Kornberg, 1977). Variable amounts of DNA ranging from 20 to 60 bp are referred to as linker DNA, which is loosely associated with another histone, H1. It is therefore only natural that either damage or repair of DNA would depend on the overall chromatin structure. For example, certain types of damage induced by chemicals are seen in the linker region (Hanawalt et al., 1979), but UV light induced thymine dimers are seen uniformly throughout the core and linker regions.

The frequency of occurrence of various types of damage in a mammalian cell is summarized in Table 2. If the rates of different events are added together, the overall rate of damage/cell/d would come to about 60,000. This is an enormous onslaught on the structural integrity of genomic machinery.

DNA Damage in Brain

There are some studies to look into the DNA damage in brain. However, these studies were conducted not with an aim to study the basic aspects of DNA damage or its repair, but to measure the accumulation of DNA-damage in this organ with respect to age. In a way, the objectives of most of these investigations appear to be checking the validity of a number of aging theories that have, as their central theme, the accumulation of genetic damage with age (Szilard, 1959; Hart and Setlow, 1974; Kirkwood and Holliday, 1979; Hayflick, 1980; Gensler and Bernstein, 1981). Thus, Price et al. (1971) have shown that SSB in DNA accumulate with age in mouse brain. The strand breaks were more in brain than in liver. They have also observed higher DNA synthesis in aging brain, possibly because the SSB can act as initiation points for DNA-synthesis. Chetsanga et al. (1977) have reported that alkaline sucrose gradient sedimentation of DNA of mouse brain showed a few bands for the old (30 mo) and only one for the young (6 mo), indicating degradation of DNA in old age owing to breaks. Murthy et al.

(1976) working with both isolated chromatin and DNA, observed more of single-strand regions in either of the preparations obtained from old rat cerebral cortex as compared to those from young.

Making use of a immunofluorescence technique where the antibody would react with only denatured or single-stranded DNA, Nakanishi and coworkers (1979) found such single-stranded regions only in old brain preparations. No significant antibody binding was noticed in the brain tissue of young animals. Moreover, no DNA damage was noticed by these workers in the epithelial cells of intestinal tract at any age. On the basis of these observations, these authors postulated that accretion of DNA damage may happen only in tissues made up of nonreplenishible cells and the same phenomenon may not be seen in such tissues made of cells with quick turnover.

This concept was indeed in line with a hypothesis advanced a few years earlier by Wheeler and Lett (1974), who proposed the possibility that the DNA-repair may be related to age in nondividing cells like neurons. These workers had subjected the DNA isolated from internal granular layer from the cerebella of beagle dogs, aged from 7 wk–13 yr, to alkaline sucrose-gradient sedimentation. From the profiles obtained, they concluded that there is an age-associated decline in the size of the DNA that can be extracted from the neuronal cells. The reduced size of DNA species in aging brain could be attributed to the increased SSB reflecting the normal aging of the cerebellum.

Mori and Goto (1982), adopting the single-strand specific S_1 endonuclease assay of Murthy et al. (1976), assessed the single-strand regions in DNA isolated from mice of different ages. Brain DNA from mice aged 15–30 mo contained 2.9% single-strand regions, whereas younger brain DNA contained only 2.0% single-strand regions. It is interesting that these workers could not find any such age-associated changes in other organs like liver, kidney, heart, and spleen.

Bergtold and Lett (1985) made use of the technique of zonal ultracentrifugation of DNA and noticed an increased number of DNA breaks with age in rabbit retinal cells. Recently Tan et al. (1990) have estimated the steady-state level of 7-methylguanine, a major product formed by methylating agents both in vitro and in vivo, but also found in untreated rodent genomes as a consequence of endogenous reactions, in male mouse brain at 11, 23, and 28 mo of age. The results showed that in nuclear DNA of brain, the levels of 7-methylguanine went up approx twofold between youth and old age.

In contrast to the above findings, a few reports have also appeared wherein no age-dependent accretion of DNA damage could be observed in brain. For example, Ono et al. (1976) failed to notice any evidence for an increased SSB in mouse cerebellar DNA with age. On the other hand, liver DNA from older mice in the same studies, was found to be fragmented and therefore smaller in size. It must, however, be noted that the authors themselves have suggested that probably the aged mice used in their study (22 mo) are not old enough compared to the age of the mice used by other workers. Very recently, Fu et al. (1991) were unable to notice any effect of age (6 vs 25 mo) or of dietary restrictions on DNA SSB in mouse brain. Two other laboratories also failed to observe any age dependent accumulation of SSB in rat brain DNA (Su et al., 1984; Mullaart et al., 1990). The reasons for this discrepancy are not known as of now.

Possible alterations at the genetic level (genetic damage) has also been examined from a different direction by some other workers. A few reports appeared indicating enhanced condensation or compaction of chromatin with age in rat brain (Kanungo and Thakur, 1979; Chaturvedi and Kanungo, 1985). Their results also showed a 50% reduction in the RNA-polymerase II activity in rat brain in old age, and the authors concluded that this may be a result of structural changes in chromatin that may occur with increasing age.

That the neuronal chromatin undergoes structural changes with age has also been observed by Berkowitz et al. (1983). Using the enzyme micrococcal nuclease as a probe for chromatin

Table 3

Template-Primer Efficiency of DNA Isolated from Young, Adult, and Old Rat Brain Tested with *E. coli* DNA-Polymerase I*

	Activity, pmoles of (^{3}H)-TMP incorporated	
	Incubation time	
DNA	15 min	45 min
Young (4 d old)	4.3	7.9
Adult (6–8 mo old)	5.7	11.2
Old (2 yr old)	7.0	17.8
"Activated" calf thymus DNA	3.7	11.4

*The reaction mixture contained, in a total volume of 50 µL, 40 m*M* Tris-HCl, pH 8.0, 1 m*M* β-mercaptoethanol, 7.5 m*M* $MgCl_2$, 4 m*M* ATP, 5 µg of either "activated" calf thymus DNA or DNA isolated from rat brain of different ages, 0.1 m*M* each of dATP, dGTP, dCTP, and 25 µ*M* dTTP (1 µci) and 0.5 U of *E. coli* pol. I (1 U is the amount of enzyme catalyzing the incorporation of 1 nmol of total nucleotides into acid insoluble product in 30 min at 37°C). At the end of the incubation, 200 µg of calf thymus DNA and 100 µg of bovine serun albumin were added as carrier and the reaction stopped by adding 1 mL of 10% cold TCA. The samples were kept in ice for 15 min and centrifuged at 400 rpm. The precipitate was washed thrice with 5% cold TCA and then with 1 mL of 95% chilled ethanol. The washed precipitate was dissolved in 0.2 mL of 0.1*N* NaOH and kept at 37°C for 1 h or at room temperature overnight. A 0.1 mL aliquot was taken into a vial containing 10 mL of Bray's mixture. Radioactivity was counted in a Beckman LS-1800 counter. Data taken from Subba Rao et al. (1992).

structure, they observed that DNA from neuronal preparation showed a decreased susceptibility to digestion during aging. In addition, there was a dramatic increase in the nucleosome spacing of the chromatin. For example, cerebral neuronal chromatin has a repeat length (nucleosome core and linker region) of 164 bp at 22 d and 11 mo, 186 bp at 24 mo, and 199 bp at 30 mo. It is supposed that such alteration in the chromatin structure may affect the RNA synthesis.

Chromatin or DNA compaction could also be studied by measuring the melting temperature (Tm) of the preparations. Kurtz and Sinex (1967) found that the Tm of the chromatin of mouse brain decreases from the third day to 13 mo of age and then increases rapidly up to 30 mo, supporting a much condensed chromatin organization in old brains. Melting curves of the isolated DNA preparation from brains of rats of different ages were also examined in our laboratory (Subba Rao et al., 1992). Young DNA samples exhibited a Tm of 79°C, and this value has increased to 81°C in the case of old preparations (>24 mo). However, the hyperchromicity decreased from 44% (young), to 37% (old). These results are interpreted as indicating that in aging rat brain some portions of the DNA are more condensed, whereas certain other portions attain a loose structure, possibly because of strand breaks. Both these possibilities were assessed subsequently in an indirect manner. When the DNA isolated from brains was used as a template-primer to *E. coli* DNA-polymerase I under appropriate conditions with one of the four deoxynucleotides being labeled, the old DNA incorporates three times more of radioactivity as compared to young (Table 3). Since strand breaks generating 3'-OH ends in DNA can serve as initiating points for the *E. coli* pol I to add deoxynucleotides using the other strand as template, the extent of radioactive incorporation could be taken as a measure of strand breaks or the number of 3'-OH groups available in the template primer. Thus, the results pointed out more strand breaks in DNA from old brains (>24 mo).

Table 4

Chromatin "Bound" and "Free" RNA Polymerases in Neuronal Nuclei Isolated from Cerebral Cortex of Adult and Old Rats*

	Total		RNA polymerase I + III		RNA polymerase II	
Age	Bound	Free	Bound	Free	Bound	Free
Adult (6 mo)	99.9 ± 5.8	332.1 ± 31.3	24.0 ± 1.4	108.9 ± 20.5	75.9 ± 7.1	223.2 ± 21
Old (2 yr)	73.4** ± 5.3	282 ± 50.2	23.3 ± 8.7	163.7** ± 13.8	50.1** ± 3.7	118.5** ± 36.4

*Values are averages ± SD from three independent experiments and represent pmoles of (^{3}H)-UMP incorporated into acid insoluble fraction/mg DNA/15 min. For more experimental details, *see* Venugopal and Subba Rao (1991).

**These values are significantly different from the corresponding values of adult at a p value of < 0.05.

Table 5

RNA Polymerase II Activity in Chromatin Isolated from Nuclei of Rat Cerebral Cortex of Different Ages—Effect of Addition of Homologous DNAs Isolated from Rat Cerebral Cortex of Different Ages*

	Chromatin	Chromatin + "young" DNA	Chromatin + "adult" DNA	Chromatin + "old" DNA
Young (10 d)	6.61 ± 1.78	9.80 (148%)** ± 1.78	7.94 (137%) ± 1.12	7.65 (115%) ± 0.35
Adult (6 mo)	9.84 ± 1.53	13.24 (135%)*** ± 1.82	11.93 (121%)** ± 1.76	11.22 (114%) ± 0.95
Old (2 yr)	7.47 ± 0.92	8.63 (115%) ± 0.65	7.84 (105%) ± 1.30	7.54 (101%) ± 1.33

*Chromatin isolated from cerebral cortex nuclei was incubated under the conditions optimal for the expression of RNA polymerase II activity. Where indicated the chromatin preparations were preincubated for 15 min with DNA (4 µg) isolated from cerebral cortex of specified age. Values are averages ± SD from three–five experiments and represent nmoles of [^{3}H]-GMP incorporated into acid insoluble fraction/mg DNA/30 min. Data reproduced from Venugopal and Subba Rao (1991).

**These values are significantly diffierent from corresponding control values at a p value < 0.1.

***This value is significantly different from the corresponding control value at a p value < 0.02.

Condensation or compaction of the genetic apparatus should result in, among other things, altered transcriptional activity. This aspect was examined recently by Venugopal and Subba Rao (1991). First, assay conditions were designed in such a way to distinguish the chromatin "bound" and "free" RNA-polymerase activities. Also, by using appropriate inhibitor (such as α-amanitin), transcriptional activity catalyzed by RNA polymerase II and by RNA polymerase I + III was separately visualized. Second, the activities were measured in nuclear fraction of different cell types (neurons, astroglia, and oligodendroglia) isolated from adult (6 mo) and old (>24 mo) rat brains. The results concerning the neuronal cells are presented in Table 4 . Both chromatin bound and free RNA-polymerase II activities were decreased with age. No age effect was seen in the activity of RNA-polymerase I + III. That the decreased RNA polymerase II activity was at least partly owing to altered chromatin was inferred by the crossmixing experiments. DNA isolated from old rat brain was unable to enhance the transcriptional activity when added to chromatin preparations obtained from brains of any age. Also, the "old" chromatin was unable to accept even the "young" (10 d) DNA as an additional exogenous template. However, when young and adult chromatins were preincubated with DNA isolated from young brain, a significant increase in transcriptional activity occurred (Table 5). It is concluded that the reduced gene expression in

Table 6A

Accumulation of DNA Damage/Alterations Found in Brain with Age

Animal	Type of damage/alteration	Reference
Mouse	Single-strand breaks	Price et al. (1971)
Mouse	Single-strand breaks	Chetsanga et al. (1977)
Mouse	Single-strand breaks	Nakanishi et al. (1979)
Mouse	Single-strand breaks	Mori and Goto (1982)
Mouse	Increase in 7-methylguanine	Tan et al. (1990)
Rat	Nuclease sensitive sites (single-strand breaks)	Murthy et al. (1976)
Rat	Increased condensation of chromatin, increased condensation as well as single-strand breaks	Kanungo and Thakur (1979); Chaturvedi and Kanungo (1985); Subba Rao et al. (1992)
Rat	Double-strand break	Hartnell et al. (1989)
Beagle dogs	Single-strand breaks	Wheeler and Lett (1974)
Rabbit	Single-strand breaks	Bergtold and Lett (1985)

Table 6B

Accumulation of DNA Damage/Alterations Not Found in Brain with Age

Animal	Reference
Mouse	Ono et al. (1976)
Mouse	Su et al. (1984)
Mouse	Fu et al. (1991)
Rat	Mullaart et al. (1990)

old brain cells is at least partly a result of altered chromatin/DNA structure. A summary of the available evidence both for and against the accumulation of DNA damage in brain with age is presented in Table 6. It is convincingly clear that the literature is overwhelmingly in favor of the conclusion that DNA damage does accumulate with the advancement of age in brain.

DNA-Repair

Just as there are a multitude of agents that attack the genetic machinery of living beings to create instability at that level, the organisms, quite naturally, have developed during the course of evolution a number of mechanisms to counteract such deleterious alterations of the genetic material. A detailed discussion of these mechanisms is beyond the scope of this article. Comprehensive reviews have appeared on the subject and the reader is referred to them (Hanawalt et al. 1979; Linn, 1982; Friedberg, 1985; Sedgwick, 1986; Collins et al., 1987; Sancar and Sancar, 1988; Friedberg, 1990,1991). However, a brief indication of the various DNA-repair systems operating in the living world is given in Table 7.

From the knowledge available today the repair events can be divided into two classes: those that simply reverse the damage without involving the breakage of phosphodiester bond, e.g., monomerization of pyrimidine dimers by an enzymatic

Table 7

Various DNA-Repair Systems in the Living World*

Mode of repair	Enzymes/proteins implicated
Reversal of damage	
Photoreactivation; demethylation	DNA-photolyase; O^6-methylguanine-methyltransferase,
Rejoining of single-strand breaks	DNA-ligase
Excision repair	
Nucleotide excision repair including mismatch repair	Poly(ADP-ribose)polymerase[1] UV-damaged DNA specific binding protein[2]; T:G mismatch specific binding protein[3]; dRPase[4]; incision endonucleases; exonuclease(s); DNA polymerase(s); DNA-ligase
Base excision repair	DNA-glycosylases; AP (apurinic/apyrimidinic) endonucleases; exonucleases; DNA-polymerase and ligase
Recombinational repair	Rec ABC gene products in *E. coli*, S_1 nuclease, single-strand binding protein, and other factors/proteins yet to be identified

*Information gathered from Linn, 1982; Friedberg, 1985; Kuenzle, 1985 and Bernstein and Bernstein, 1991. Specific references cited in Arabic numbers are as follows: [1]Satoh and Lindahl, 1992;[2] Chao et al., 1991; [3]Heywood and Burke, 1990; [4]Price and Lindahl, 1991.

reaction dependent on light of wavelength more than 300 nm, removal of methyl groups, and simple rejoining of the strand breaks. The other category of DNA-repair processes is the nucleotide and base excision repair. Except for the initial difference, these two processes actually constitute a common pathway (Fig. 5).

There is yet a third type of DNA repair, the recombinational repair. The precise enzymology of this form of repair is less understood. Also, this mode of DNA-repair is of no significant relevance to a postmitotic cell like neuron since the recombination repair is essentially a postreplicational event to take care of the possible errors introduced during active DNA synthesis.

The first step in nucleotide excision repair pathway sees to be the recognition and incision of the damaged site by an incision endonuclease. From the plethora of endonucleases found in different organisms, it appears that these enzymes have rather broad and varied specificities (Linn, 1982; Lambert et al., 1988). Some recent information suggests that the recognition of the site by the endonuclease is facilitated by the binding of damage specific proteins. For example, a protein that has specific affinity for UV-damaged DNA has been isolated from HeLa cells (Chao et al., 1991). Similarly, Satoh and Lindahl (1992) have shown that the enzyme poly(ADP-ribose)polymerase has the capacity to recognize and attach itself to a damaged site in the DNA molecule. This attachment activates the synthesis of polyADP-ribose, resulting in autopolyADP-ribosylation of the enzyme. This automodified enzyme has reduced affinity for DNA and is therefore released, making the damaged site accessible to DNA-repair enzymes. In the same line, specific T:G mismatch binding proteins in mammalian cells are also reported (Heywood and Burke, 1990). Further, in *E. coli*, an enzyme capable of specifically nicking one strand of the duplex next to the T at a mismatched T:G has been demonstrated (Hennecke, et al., 1991).

The second step is the excision of the damaged region, which may include some adjacent nucleotides as well, by an exonuclease. The third step involves the filling up of the gap by a DNA-polymerase using the other strand as template, and finally the sealing of the gap by DNA-ligase. In the case of base excision repair, first the base-

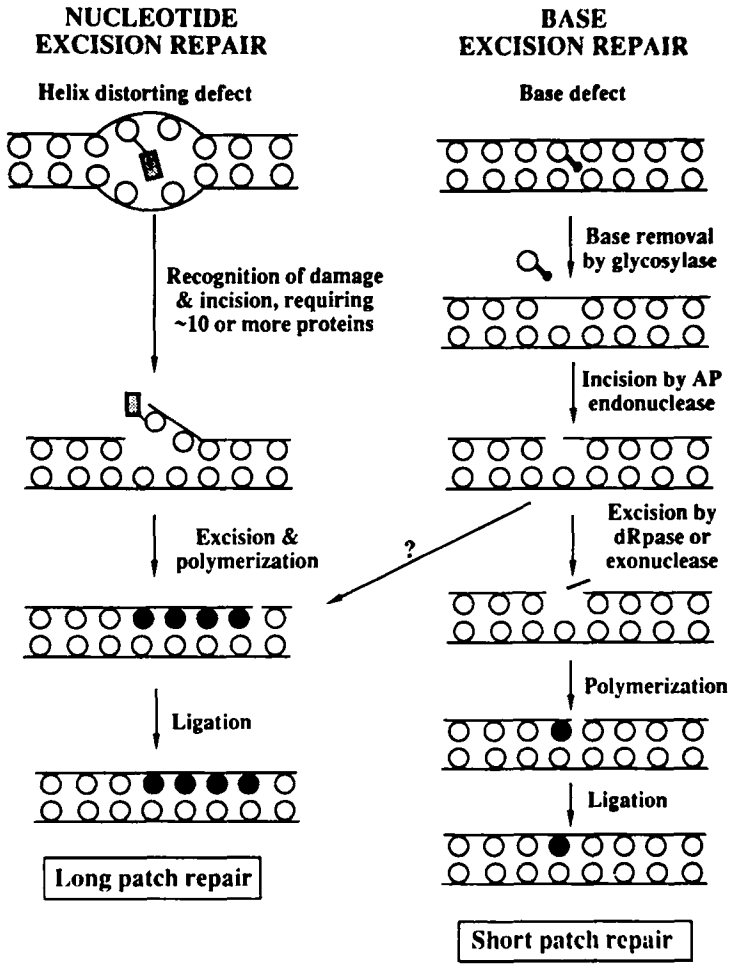

Fig. 5. An outline of excision repair pathway: On the left side is nucleotide excision repair, and on the right is the base excision repair. This diagram is kindly provided by Larry H. Thompson of Lawrence Livermore National Laboratory, Livermore, CA.

less site formed either spontaneously or by the action of DNA-glycosylase would be recognized by an apurinic/apyrimidinic endonuclease and the site incised. Henceforth, the subsequent steps are similar to that of nucleotide excision repair. Although until now it was assumed that the excision of base-free sugar phosphate residue from apurinic/apyrimidinic sites is carried out by an exonuclease, it is now shown that this step is catalyzed by a specific enzyme deoxyribophosphodiesterase (dRpase) both in *E. coli* and mammalian cells (Price and Lindahl, 1991).

It is generally believed that the nucleotide excision repair is characterized by what is described as "long patch" repair based on the number of nucleotides incorporated (about 100) per each repair segment, whereas in the base excision repair the patch is a short one comprising only three to four nucleotides (Fig. 5).

Much of the information about the enzymology of DNA-repair has emanated from prokaryotic systems and in particular the *E. coli*. A large number of enzymes involved in different repair pathways have been well studied in *E. coli*, but

exact information about similar enzymes from mammalian cells is scanty. Comparable information from brain is even scantier (Linn, 1982; Friedberg, 1985,1990; Subba Rao, 1990). Although it is generally believed that the DNA-repair pathways in mammalian and human cells are similar to those observed in prokaryotes, evidence is mounting to indicate that in these higher systems the mechanism may be more complicated. If one considers only the initial step of damage recognition (for example, UV-induced pyrimidine dimers) the number of genes involved tends to increase from prokaryotes to humans. Thus, in T4 infected *E. coli*, only a single T4 coded gene (den V gene) is required for the incision. However, in uninfected *E. coli*, four genes, the UVR ABC and D are required to achieve the same process. In mammalian cells including human there are indications to suggest that at least 15 gene products are required in the incision process itself (Fischer et al., 1985, Hoy et al., 1985; Cleaver and Karentz, 1986). A number of human repair genes are being cloned and their precise functions looked into in different laboratories (Thompson, 1989).

The subject of DNA-repair has taken a new turn with the discovery by Hanawalt and coworkers (Hanawalt, 1987) that there exists a genomic heterogeneity in DNA-repair process—meaning that damages in certain regions of the genome may be repaired preferentially. With techniques developed to quantify UV-induced pyrimidine dimers in specific fragments of genomic DNA and also to assess the rates of DNA-repair in different genomic domains of the same cell, this group showed that although the overall dimer removal is poor in Chinese hamster ovary cells (CHO), the repair of dimers in the restriction fragment of active and essential dihydrofolate reductase gene is very efficient (Mellon et al., 1987). This gave the important clue that damage in actively transcribing genes is repaired preferentially. Studies further demonstrated that in mouse cells, the expressed c-abl protooncogene is preferentially repaired, whereas the unexpressed c-mos protooncogene is poorly repaired (Madhani et al., 1986) and also some types of damage, such as interstrand crosslinks, are repaired more efficiently than other lesions, like psoralen photomonoadducts (Vos and Hanawalt, 1987).

In Table 8, some enzymes, which are either proven or suspected to participate in the DNA-repair process and to be present in brain tissue, are listed. In spite of the reasonably impressive number of enzymes found in brain, a systematic examination of the activities with reference to age seems to have been carried out only in a couple of cases. These will be mentioned below along with discussion on DNA-repair in brain.

DNA-Repair in Brain

There are some studies that examined the changes in DNA-repair potential of brain with age. It was probably Alexander (1967) who first noticed that DNA-repair system is at a low key once cells are differentiated into a postmitotic state. A number of subsequent observations confirmed this postulation with special reference to brain cells (Bernstein and Bernstein, 1991). Nonetheless, results from different laboratories also revealed that repair is not completely shut off but only low in adult brain. For instance, Korr and Schultz (1989) have demonstrated through autoradiography, low but significant levels of DNA-repair in various types of cells of adult mouse brain in vivo. This is in line with the earlier observations of Waser et al. (1979) and of Subba Rao and Subba Rao (1984), who reported significant levels of DNA-polymerase β, generally considered to be a repair enzyme, in adult and aging brain. That not only the DNA-polymerase β levels are significant in adult and old mouse brain but also no change occurs in the fidelity of this enzyme between young, adult, and old ages has been demonstrated by Subba Rao et al. (1985). Also, the nonspecific alkaline DNase of rat brain, a putative "housekeeping" DNA-repair enzyme was found to exhibit high activity during adult and old ages (Subba Rao and Subba Rao, 1982; Subba Rao, 1990). Furthermore, Jensen and Linn

Table 8

DNA-Repair Enzymes in Brain*

Enzyme	Mechanism of action	Reference
Endonuclease of lamb brain	Attacks single-stranded DNA releasing oligonucleotides with 5'-phosphate	Healy et al. (1963)
Acid and alkaline DNases of rat brain	Acid DNase attacks native DNA releasing oligonucleotides with 3'-phosphate; alkaline DNase degrades single-stranded DNA releasing 5'-phosphate oligonucleotides	Sung (1968)
Acid DNase (UV DNase) and alkaline DNase (AP DNase) from chick and rat brain	Acid DNase (UV DNase) attacks native DNA or UV irradiated native DNA; alkaline DNase (AP DNase) degrading DNA with no specificity but exhibiting higher activity toward damaged (depurinated) DNA. Both enzymes act as endonucleases	Subba Rao, (1990); Rajagopal and Subba Rao (1992)
AP-endodeoxy nuclease from rat neocortex chromatin	Incises near AP sites of supercoiled DNA	Ivanov et al. (1988)
Exonuclease (DNase B III) from rat brain neuronal nuclei	Excises single-strand DNA of single-strand termini in a duplex DNA in 5' → 3' direction. Nucleoside, 5'-monophosphate is released	Ivanov et al. (1983)
Uracil-*N*-Glycosylase of human fetal brain	Releases uracil from DNA specifically	Krokan et al. (1983)
DNA-polymerase β of rat and mouse brain	Polymerization of deoxynucleoside triphosphates	Hubscher et al. (1979)
DNA-ligase of neuronal and glial cells of guinea pig brain and rat cerebellum	Joining the DNA strands utilizing ATP	Inoue and Kato, (1980)
Poly (ADPR) synthase of bovine brain	Catalyzes the synthesis of acceptor (auto) bound poly ADP-ribose	Bilen et al. (1981)
O^6-alkyl-guanine-DNA-alkyl transferase of rat and human brain	Removes the methyl/alkyl group from O^6-methylguanine in DNA and transfers it to one of its own cysteine residues	Wiestler et al. (1984)
Photolyase of marsupial brain	Monomerizes the pyrimidine dimers	Rupert (1975)

*Part of the information is gathered from Linn, 1982; Friedberg, 1985; and Kuenzle, 1985.

(1988) noticed that when human neuroblastoma cells differentiate in culture to a postmitotic state, the levels of DNA-polymerase β and uracil DNA-glycosylase remain unchanged, whereas AP endonuclease activity actually shot up threefold. Recently, Subrahmanyam and Subba Rao (1991) examined the DNA-repair capacity, as judged by the incorporation in vitro of (^{3}H)-thymidine into DNA of isolated neuronal cells, as a function of age. The results point out that whereas the repair potential decreases markedly from young (10 d) to adult (6 mo), no further decrease between adult and old ages (>540 d) was noticed (Table 9). It does appear that adult and even aging brain possesses the necessary machinery to take care of at least some forms of DNA damage.

That not only significant DNA-repair process occurs in a model neuronal cell system but also that the tenet of genomic heterogeneity of DNA-repair is applicable to this postmitotic system as

Table 9

UV-Induced Unscheduled DNA Synthesis in Neurons of Rat Brain at Different Ages*

Age	Control	$20J/m^2$	Ratio UV/control	$40J/m^2$	Ratio UV/control
Young (1 d old)	61 + 2 (8)	71 + 6** (8)	1.16	91 + 21*** (3)	1.49
Adult (6 mo old)	28 + 4 (8)	35 + 2** (8)	1.25	33 + 2*** (4)	1.18
Old (>540 d old)	29 + 3 (8)	30 + 4 (8)	1.03	34 + 3*** (3)	1.17

*Values are expresed as mean of DPM $\times 10^{-3}$ of [^{3}H]thymidine incorporated/mg DNA + SD. Number in parentheses indicates the number of experiments carried out. For other details please *see* Subrahmanyam and Subba Rao (1991).

**These values are significantly different from the corresponding control values $p < 0.001$.

***These values are significantly different from the corresponding control values $p < 0.05$.

well, has been elegantly shown by Hanawalt and colleagues in the recent past (Hanawalt et al., 1992). Normally, proliferating cells isolated from rat pheochromocytoma (PC12 cells) serve as a model for neuronal cells since they can be induced by nerve growth factor (NGF) to differentiate to attain many of the physiological properties unique to neuronal functions. Once this differentiation was initiated, it was found that the overall DNA-repair efficiency in response to UV-irradiation dropped from about 15% to an undetectable level. One gene that is known to become active during NGF-induced differentiation is that of a 43-kDa growth-associated phosphoprotein, known as GAP-43. This protein has a role in establishing the neural network. Using a cDNA clone for this gene as a probe for quantitative hybridization analyses of Southern blots, these workers found that 22–30% of the UV-induced pyrimidine dimers were removed within 48 h after the UV exposure of proliferating cells; however, this was increased to 45–55% in the completely differentiated cells.

Another gene, that of synapsin-I, is known to be actively expressed throughout the functional lifetime of a neuron. Following the same strategy as in the case of GAP-43, Hanawalt et al. (1992) found that 70–80% of the pyrimidine dimers from both proliferating and differentiated cells were removed within 48 h even though bulk DNA-repair levels were negligible in the differentiated postmitotic cells. However, no evidence for transcribed strand specific repair could be seen in the postmitotic cells, which is in contrast to what has been noticed with proliferating cell systems. Nevertheless, it is clear that the active gene specific repair occurs in nondividing neuron type cells.

A limited number of laboratories did take up the study of DNA-repair in brain to see whether any correlation could be found between aging and DNA-repair. The results are conflicting. Thus, Wheeler and Lett (1974), although finding no deterioration in the capacity of beagle dog cerebellar internal granular layer neurons to join the single-strand breaks induced by gamma radiation, also found that there was an age-associated decline in the size of the DNA that can be extracted from the cells. If the decrease in size can be interpreted as increased strand breaks, then it is not clear how the cells exhibit the same rejoining capacity at all ages. It has to be assumed that the cells are able to join the gamma-ray-induced breaks but not other types of breaks. Thus the results can still be interpreted as a decline in some form of DNA-repair in these cerebellar neurons with age.

DeSousa et al. (1986), working with mouse dorsal root ganglia neurons in culture, measured UV-induced unscheduled DNA synthesis (UDS) as a function of age of the animal. In both strains of mice used, significant decrease in UDS was found in old age as compared to the adult. It is interesting that in the same study no age related decrease in UDS was noticed in lymph node cells.

Table 10

Changes in DNA-Repair Potential of Brain with Age

Reports in which a decrease was found	Observation
Wheeler and Lett (1974)	Decreased repair of spontaneous single-strand breaks in beagle dog cerebellar neurons
DeSousa et al. (1986)	Decreased UV-induced UDS in mouse dorsal root ganglia
Subrahmanyam and Subba Rao (1991)	Decreased UV-induced UDS in rat cerebral cortex neurons
Reports in which no decrease was found	
Wheeler and Lett (1974)	No change in the capacity of rejoining of gamma ray-induced strand breaks in beagle dog cerebellar neurons
Ono and Okada (1978)	No change in gamma ray-induced strand break repair in mouse cerebellum
Gensler (1981)	No change in UV-induced excision repair in hamster whole brain

Subrahmanyam and Subba Rao (1991) recently examined the usefulness of isolated neurons from rat brain cerebral cortex as a model system to study DNA-repair without resorting to the use of hydroxyurea to inhibit the possible replicative DNA synthesis that might be going on simultaneously in a given cell system. The results indicated that neurons obtained from adult (6 mo) and old (>540 d) animals offer a good model system to measure the UV-induced UDS without any interference of DNA-replicative synthesis. These investigations also revealed that although the spontaneous UDS in old neurons remains unchanged as compared to the adult level, the response of aging neurons, in contrast to the young and adult neurons or spleenic lymphocytes of any age, to a mutagenic challenge like UV light is markedly limited (Table 9). It is suggested that it is this lack of responsive DNA-repair against a given damage that may lead to general metabolic deterioration and senescence.

The evidence for and against an age-dependent decline in DNA-repair capacity in brain is summarized in Table 10. Unlike the case of DNA-damage (Table 6), an equal number of reports exist on either side. However, a closer examination of the data would reveal that those studies that could not find any decline of DNA-repair with age are not strictly comparable to others. In the mouse study by Ono and Okada (1978) gamma-ray-induced strand breaks were followed rather than UV-induced damage in dorsal root ganglia, as is the case with DeSousa et al. (1986). Similarly, the hamster studies of Gensler (1981) have been carried out with whole brain rather than a specific neuronal population. It would therefore appear that measurement of DNA-repair, in contrast to DNA-damage, is not a straightforward issue. Comparisons of data should perhaps be made only when the repair of a particular type of DNA damage in a specific preparation of brain was measured and in an identical manner, especially in view of the predicted complexities of DNA-repair pathways in a highly evolved organ like brain. Taking all these aspects into consideration it is still tempting to uphold the DNA damage and repair theory of aging.

Some evidence to strengthen the above contention has actually emerged from the studies of Niedermuller (1985) and of Washington et al. (1989). The former has studied repair of four different types of DNA damage (UDS after treatment with DNA-damaging agent, *N*-nitrosomethylurea, after methylmethanesulfonate damage SSB repair measured through nucleoid sedimentation, repair of double-strand breaks determined by neutral elution, and removal of endonuclease-sensitive sites as measured by velocity sedimentation in alkaline sucrose gradient) in different tissues of rat. The first type of repair declined with age in all the tissues including the brain. Single-strand

Table 11

Genetic Disorders that Show Signs of Elevated Genomic (DNA) Damage/Premature Aging/Neurodegeneration*

Disorder	Symptoms		
	Genomic damage	Neurodegeneration	Premature aging
Ataxia telangiectasia (Louis-Bar syndrome)	+	+	+
Cockayne's syndrome	+	+	+
Down's syndrome	+	+	+
Xeroderma pigmentosum	+	+	–
Huntington's disease	+	+	–
Parkinson's disease	+	+	–
Alzheimer's disease	+	+	–
Friedrich's ataxia	+	+	–
Amyotrophic lateral sclerosis	+	+	–
Familial dysautonomia	+	+	–
Usher's syndrome	+	+	–
Bloom's syndrome	+	–	–
Fanconi's anemia	+	–	–
Werner's syndrome	+	–	+
Progeria (Hutchinson-Gilford's syndrome)	?	–	+
Turner's syndrome	–	–	+

*Information gathered from Friedberg, 1985; Warner and Price, 1989; Bohr et al. 1989; Bernstein and Bernstein, 1991. Question mark indicates lack of unequivocal information.

repair was most significantly reduced only in testis and brain of 28-mo-old rat. No change was found in that case of DSB repair. The ability to remove endonuclease-sensitive sites was markedly lost with age in brain apart from other tissues. The activities of 3-methyladenine-DNA-*N*-glycosylase (MAG) and O^6-methylguanine-DNA-methyltransferase (MGMT) were measured in mouse brain by Washington et al. (1989). No change was noticed in the case of MGMT, but significant lowering of MAG activity was observed with age.

Proof of a cause-and-effect relationship in biological phenomena is often facilitated by the study of genetic mutants, and, in the case of humans, genetic disorders. Accordingly, a search was made, over the years, for genetic disorders characterized by premature aging. If DNA damage and repair has anything to do with aging it should be evidenced in such individuals. Martin (1978) listed 162 genetic syndromes in humans with some or many signs of premature aging. About 21 features are considered as markers for accelerated aging. Those aspects along with possible accumulation of diverse forms of DNA damage and decline in DNA-repair capacity in some of these syndromes have been discussed in detail by Friedberg (1985), Warner and Price (1989), Bohr et al. (1989), and by Bernstein and Bernstein (1991). What calls for attention is the fact that many of these genetic disorders also display signs of mental dysfunction. In Table 11 are listed 16 human syndromes and 15 of them show a common feature of elevated genomic damage of some kind. It is unlikely to be a mere coincidence that a large number of them (11 out of 16) suffer from neurodegeneration, whereas 6 of them reveal striking signs of premature aging as well.

However, only those genetic disorders that exhibit premature aging, neurodegeneration (mental defects), and some form of chromosomal/DNA damage all together will be empha-

sized here. Perhaps the most appropriate disorder under this category is Down's syndrome. It has several features of premature aging and the genetic defect is trisomy of the distal part of the long arm of chromosome 21. The critical segment of chromosome 21 is shown to have three genes coding for copper- and zinc-dependent superoxide dismutase, oncogene ets-2, and cystathione β-synthase (Delabar et al., 1987). Since elevated levels of superoxide dismutase are found in various tissues of these individuals, it is postulated that the accelerated aging of these patients may be caused by overproduction of superoxide dismutase, which is responsible for the production of H_2O_2 while scavenging the oxygen-free radicals. The brains of Down's syndrome individuals are particularly vulnerable to oxidative DNA damage because the high levels of superoxide dismutase found in this tissue are not accompanied by an elevation in the glutathione peroxidase and catalase (Balazs and Brookshank, 1985) that would have normally helped in removing the overproduced H_2O_2. Other genetic syndromes characterized by signs of nervous debility, premature aging, and DNA damage/decreased DNA-repair capacity, are Ataxia Telangiectasia (AT) and Cockayne syndrome (CS).

Cells from AT patients are hypersensitive to X-ray irradiation (Mckinnon, 1987) but not to UV light (Lehmann et al., 1977). The precise biochemical defect behind this hypersensitivity toward X-ray is not known at this time. On the other hand, CS, with its onset at about 2 yr of age and with many symptoms of premature aging and mental retardation, is characterized by extreme sensitivity to UV irradiation (Schmickel et al., 1977). However, CS cells have been found to carry out UV-induced excision repair with normal efficiency (Ahmed and Setlow, 1978). This led Mayne and Lehmann (1982) to suspect, on the basis of the observed failure of recovery of RNA synthesis in CS cells after UV irradiation, that the excision repair defect in this syndrome is confined to the actively transcribing DNA strand. Thus, this disorder may have a mutation in some gene that is normally involved in the specific preferential repair of active genes. Evidence to support this idea has subsequently come from Mayne et al. (1988). This biochemical defect in CS cells serves as a reminder to underline the enormous complexities of DNA repair mechanisms in higher systems and also to the caution that must be exercised in comparing the DNA-repair results from one system to the other.

It is not clear why symptoms of accelerated aging are not apparent in all the syndromes that show genomic damage, if these two features are related. In this context, it is argued (Bernstein and Bernstein, 1991) that most of these genetic defects (with the exception of Bloom's syndrome and Fanconi's anemia) can be considered as segmented progerias since they exhibit some, but not all, features of premature aging (*see* Martin, 1978 for details of aging symptoms).

It thus appears that there is more than a casual link among genomic damage, aging, and neurodegeneration. Here the word "aging" may have to be interpreted as either signs of premature aging or increased vulnerability of the individual for certain diseases with advancement of age.

Discussion and Future Perspectives

A large volume of literature has gathered depicting the aim of checking the validity of DNA damage and repair hypothesis of aging. However, these efforts have not yielded the much-needed proof for the hypothesis. Unfortunately, the contrary also cannot be concluded with definitive evidence. Much of this confusion appears to stem from the complexity of DNA damage, the repair mechanisms, the methodologies adopted to measure these parameters, the type of tissue or cells used in various studies, and finally the animal species used in the experimentation.

From the foregoing discussion, it can be inferred that a postmitotic tissue like brain, where the cells have lost their potential to divide but are endowed with high metabolic activity, is one of the most appropriate systems to examine

genomic damage and its repair as a function of age. From the relatively small number of reports that exist on brain, it is seen that the data are less conflicting and generally support an age-related accumulation of genomic damage (Table 6). Whether this cumulative genetic instability is owing to impaired repair mechanisms is far from clear, as the results regarding the DNA repair potential of brain in relation to age are conflicting and even confusing (Table 10).

Neurons are characterized by tremendous physiological activity, which includes a high level of gene expression. As against 5 to 10% in other tissues, about 30% of the genomic DNA is transcribed in brain cells. Transcriptional activity is two to three times more in neurons as compared to cells in other organs (Tobin and Khrestchatisky, 1989). Complexity of mRNA species in brain is much more and several thousands of unique and brain-specific mRNA species, both Poly A^+ and Poly A^-, are reported (Chaudhari and Hahn, 1983). Moreover, most of the rare complex class mRNA species in brain are present at much less than one copy per cell (Chikaraishi, 1979). Very recently, Eberwine et al. (1992), using a novel RNA amplification technique in a single neuron, have shown that each single neuron can be distinguished at the molecular level by its mRNA complexity.

From a different direction, it is becoming increasingly apparent that DNA-repair process is not homogeneous and lesions in actively transcribing genes are preferentially repaired both in proliferating and nondividing cell systems (Bohr and Wasserman, 1988; Hanawalt, 1989, Hanawalt, 1992; *see also* discussion under the section "DNA-Repair"). The studies of Mellon and Hanawalt (1989) support a functional coupling between DNA-repair and transcription: induction of the *E. coli* lactose operon selectively increases repair of its transcribed strand. In a model neuronal cell system, the active gene specific repair is confirmed but then, postmitotic cells appear to affect the repair in both the strands and no preference toward transcribing strand was noticed (Hanawalt et al., 1992). In a quiescent cell population, it may be more advantageous to correct the damage in both the strands with equal efficiency since the nontranscribed strand is needed to serve as template for the excision-repair process.

Under the circumstances, it would be most logical to expect that neurons, with high transcriptional activity and with a number of single copy essential genes, would maintain high and efficient DNA-repair mechanisms in order to keep up the fidelity of vital processes like transcription and translation throughout the life-span of the species.

It is, however, intriguing that in adult brain, DNA-repair capacity is barely detectable, but it is also seen that this low activity is stimulated by mutagenic challenge in an age-dependent manner (Subrahmanyam and Subba Rao, 1991). It is tempting to speculate that DNA-repair must be a highly discrete process in brain. For example, since replication has no relevance to neuronal cells, any lesion that could interfere with transcriptional process may be removed immediately, whereas other lesions elsewhere might be ignored. It is possible that even among a set of genes undergoing transcription at a given point of time, there may be a controlled kinetic preference to remove damage from some of the more essential genes almost immediately. With time, the preference may switch over to other active genes. But for such a highly controlled and subtle mechanism, it would be difficult to rationalize the normally very low DNA-repair activity observed in these cells.

The situation being thus, it should be most rewarding to examine the repair of a given site specifically altered gene introduced into the neuronal cell population. The available technology would make such studies possible. It may very well provide, apart from some vital information about the DNA-repair pathways in a postmitotic cell, definitive answers about the DNA-repair potential being the longevity assurance system in a given species. This may well constitute a major direction for future neurogerontological research. It is the hope that this review will spark such interest.

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References

Ahmed F. E. and Setlow R. B. (1978) Excision repair in Ataxia Telangiectasia, Fanconi's Anemia, Cockayne Syndrome and Bloom's syndrome after treatment with ultraviolet radiation and *N*-acetyl-2-acetylaminofluorine. *Biochim. Biophys. Acta* **521,** 805–817.

Alexander P. (1967) The role of DNA lesions in processes leading to aging in mice. *Symp. Soc. Exp. Biol.* **21,** 29–50.

Ames B. N. (1983) Dietary carcinogens and anticarcinogens. *Science* **221,** 1256–1264.

Balazs R. and Brookshank B. W. (1985) Neurochemical approaches to the pathogenesis of Down Syndrome. *J. Ment. Defic. Res.* **29,** 1–14.

Barrows L. R. and Magee P. N. (1982) Nonenzymatic methylation of DNA by S-adenosyl methionine in vitro. *Carcinogenesis* **3,** 349–351.

Bergtold D. S. and Lett J. T. (1985) Alterations in chromosomal DNA and aging: An overview, in *Molecular Biology of Aging: Gene Stability and Gene Expression* (Sohol R. S., Birnbaum L. S., and Cutler R. G., eds.), Raven, New York. pp. 23–26.

Berkowitz E. M., Sanborn A. C., and Vaughan D. W. (1983) Chromatin structure in neuronal and neuroglial cell nuclei as a function of age. *J. Neurochem.* **41,** 516–523.

Bernstein C. and Bernstein H. (1991) *Aging, Sex and DNA-Repair.* Academic, San Diego, CA.

Bilen J., Ittel M. E., Nidergang C., Okazaki H., and Mandel P. (1981) Poly (Adenosine diphosphate ribose) polymerase activity in neuronal and glial nuclei from bovine cerebrum. *Neurochem. Res.* **6,** 1253–1263.

Bohr V. A. and Wassermann K. (1988) DNA-repair at the level of the gene. *Trends Biochem. Sci.* **13,** 429–433.

Bohr V. A., Evans M. K., and Albert J. F., Jr. (1989) DNA repair and its pathogenic implications. *Laboratory Investigation* **61,** 143–161.

Brown T. C. and Jiricny J. (1987) A specific mismatch repair event protects mammalian cells from loss of 5-methyl cytosine. *Cell* **50,** 945–950.

Chao C. K. C., Huang S. L., and Lin-Chao S. (1991) Ca^{2+}-mediated inhibition of a nuclear protein that recognizes UV-damaged DNA and is constitutively overexpressed in resistant human cells: DNA-binding assay. *Nucl Acids Res.* **19,** 6413–6418.

Chaturvedi M. M. and Kanungo M. S. (1985) Analysis of conformation and function of the chromatin of the brain of young and old rats. *Mol. Biol. Rep.* **10,** 215–219.

Chaudhari N. and Hahn W. A. (1983) Genetic expression in the Developing Brain. *Science* **220,** 924–928.

Chetsanga C. J., Tuttle M., Jacobini A., and Johnson C. (1977) Age associated structural alterations in senescent mouse brain DNA. *Biochem. Biophys. Acta* **474,** 180–187.

Chikaraishi D. M. (1979) Complexity of cytoplasmic polyadenylated and non polyadenylated rat brain ribonucleic acids. *Biochemistry* **18,** 3249–3256.

Cleaver J. E. and Karentz D. (1986) DNA-repair in man: Regulation by a multigene family and association with human disease. *BioEssays* **6,** 122–127.

Cole R. (1970) Light induced cross-linking of DNA in the presence of a furocoumarin (Psoralen) studies with phage λ, *E. coli* and mouse leukemia cells. *Biochim. Biophys. Acta* **217,** 30–39.

Collins A., Johnson R. T., and Boyle J. M., eds. (1987) Molecular biology of DNA-repair. *J. Cell. Science,* Supplement 6, The Company of Biologists Limited, Cambridge, UK.

Cutler R. G. (1991) Human longevity and aging: possible role of reactive oxygen species. *Ann. NY Acad. Sci.* **621,** 1–28.

Delabar J. M., Sinet P. M., Chadefaux B., Nichole A., Gegonne A., Stehelin D., Fridlansky F., Crean Goldberg N., Turlsan C., and de Grouchy J. (1987) Submicroscopic duplication of chromosome 21 and trisomy 21 phenotype (Down Syndrome). *Hum. Genet.* **76,** 225–229.

De Sousa J., DeBoni U., and Cinader B. (1986) Age-related decrease in ultraviolet induced DNA-repair in neurons but not in lymph node cells of inbred mice. *Mech. Aging Dev.* **36,** 1–12.

Dobbing J. (1971) Undernutrition and the developing brain: The use of animal models to elucidate the

human problem, in *Chemistry and Brain Development* (Paoletti R. and Davision A. N., eds.), Plenum, New York, London, pp. 399–412.

Eberwine J., Yeh H., Miyashiro K., Cao Y., Nair S., Finnel R., Zettel M., and Coleman P. (1992) Analysis of gene expression in single live neuron. *Proc. Natl. Acad. Sci. USA* **89,** 3010–3014.

Fischer E., Keijzer W., Thielmann H. W., Popando 0., Bohvert E., Edler L., Jung, E. G., and Bootsma, D. (1985) A ninth complementation group in Xeroderma pigmentosum, XP1. *Mutation Res.* **145,** 217–225.

Friedberg E. C. (1985) *DNA-Repair.* Freeman and Company, New York.

Friedberg E. C., ed. (1990) The enzymology of DNA-repair. *Mutation Res.* **236,** 145–312.

Friedberg E. C. (1991) Eukaryotic DNA-repair: Glimpses through the yeast *Saccharomyces Cerevisiae. BioEssays* **13,** 295–302.

Fu C. S., Harris S. B., Wilhelm P., and Walford R. L. (1991) Lack of effect of age on dietary restriction on DNA single stranded breaks in brain, liver and kidney of (C3H × C57BL/10) F_1 mice. *J. Gerontol.* **46,** B78–80.

Gensler H. L. (1981) Low levels of UV induced UDS in post-mitotic brain cells in hamsters: Possible relevance to aging. *Exp. Gerontol.* **16,** 199–207.

Gensler H. L. and Bernstein H. (1981) DNA-damage as the primary cause of aging. *Q. Rev. Biol.* **56,** 279–303.

Hanawalt P. C. (1987) On the role of DNA damage and repair processes in aging: Evidence for and against, in *Modern Biological Theories of Aging* (Warner H. R. Butler R. N., Sprott R. L., and Schneider E. L., eds.), Raven, New York, pp. 183–198.

Hanawalt P. C., Cooper P. K., Ganesan A. K., and Smith C. A. (1979) DNA-repair in bacteria and mammalian cells. *Ann. Rev. Biochem.* **48,** 783–836.

Hanawalt P. C. (1989) Concepts and models for DNA repair: From *Escherichia coli* to mammalian cells. *Environ. Mol. Mutagen.* **14,** 90–98.

Hanawalt P. C., Gee P., Ho L., Hsu R. K., and Kane C. J. M. (1992) Genomic heterogeneity of DNA repair. *Ann. NY Acad. Sci.* in press.

Harman D. (1981) The aging process. *Proc. Natl. Acad. Sci. USA* **78,** 7124–7128.

Hariharan P. V. and Cerutti P. A. (1977) Formation of products of the 5,6-dihydroxydihydrothymine type by ultraviolet light in hela cells. *Biochemistry* **16,** 271–275.

Hart R. W. and Setlow R. B. (1974) Correlation between deoxyribonucleic acid excision repair and life span in a number of mammalian species. *Proc. Natl. Acad. Sci. USA* **71,** 2169–2173.

Hartnell J. M., Storrie M. C., and Mooradian, D. (1989) The tissue specificity of the age related changes in alkali induced DNA unwinding. *Mutation Res.* **219,** 187–192.

Hartwig A. and Beyersmann D. (1989) Comutagenicity and inhibition of DNA repair by metal ions in mammalian cells. *Biol. Trace Elem. Res.* **21,** 359–365.

Haynes R. H. (1988) Biological context of DNA repair, in *Mechanisms and Consequences of DNA Damage Processing* (Friedberg E. C. and Hanawalt P. C., eds.), Liss, New York, pp. 577–584.

Hayflick, L. (1980) Recent advances in the cell biology of aging. *Mech. Aging Dev.* **14,** 59–79.

Healy J. W., Stollar D., Simon M. I., and Levine L. (1963) Characterization of phosphodiesterase from lamb brain. *Arch. Biochem. Biophys.* **103,** 461–468.

Hennecke F., Kolmar H., Brundl K., and Fritz H. J. (1991) The Vsr gene product of *E. coli* K-12 is strand and sequence specific DNA mismatch endonuclease. *Nature* **353,** 776–778.

Heywood L. A. and Burke J. F. (1990) Mismatch repair in mammalian cells. *BioEssays* **12,** 473–477.

Hoy C. A., Thompson L. H., Mooney C. L., and Salazar E. P. (1985) Defective DNA-cross link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. *Cancer Res.* **45,** 1737–1743.

Hubscher U., Kuenzle C. C., and Spadari S. (1979) Functional roles of DNA-polymerase β and γ. *Proc. Natl. Acad. Sci. USA* **76,** 2316–2320.

Inoue I. and Kato T. (1980) Nuclear DNA-ligase and its action on chromatin DNA in neuronal, glial and liver nuclei isolated from adult guinea pigs. *J. Neurochem.* **34,** 1574–1583.

Ivanov V. A., Goziev A. I., and Tretyak T. M. (1983) Exodeoxyribonuclease from rat brain specific for single stranded DNA. *Eur. J. Biochem.* **137,** 517–522.

Ivanov V. A., Tretyak T. M., and Afonin Y. N. (1988) Excision of apurinic and/or apyrimidinic sites from DNA by nucleolytical enzymes from rat brain. *Eur. J. Biochem.* **172,** 155–159.

Iyer V. N. and Szybalski W. (1963) A molecular mechanism of mitomycin action: Linking of complimentary DNA strands. *Proc. Natl. Acad. Sci. USA* **50,** 355

Jensen L. and Linn S. (1988) A reduced rate of bulky DNA adduct removal is coincident with differentiation of human neuroblastoma cells induced by nerve growth factor. *Mol. Cell. Biol.* **8,** 3964–3968.

Kanungo M. S. and Thakur M. K. (1979) Modulation of acetylation of histones and transcription of chromatin by butyric acid and 17β-estradiol in the brain of rats of various ages. *Biochem. Biophys. Res. Commun.* **87,** 266–271.

Kasai H., Crain P. F., Kuchino Y., Nishimura S., Ootsuyama A., and Tanooka H. (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7,**1849–1851.

Kirkwood T. B. L., and Holliday R. (1969) The evolution of aging and longevity. *Proc. R. Soc. London,* **B205,** 531–546.

Korr H. (1980) *Advances in Anatomical and Embryological Cell Biology, vol. 61: Proliferation of Different Cell Types in the Brain.* Springer-Verlag, Berlin, pp. 5–16.

Korr H. and Schultz B. (1989) Unscheduled DNA-synthesis in various types of cells of the mouse brain *in vivo. Exp. Brain Res.* **74,** 573–578.

Kornberg R. D. (1977) Structure of chromatin. *Ann. Rev. Biochem.* **46,** 931–954.

Kittler L and Lober G. (1977) Photochemistry of nucleic acids. *Photobiol. Rev.* **2,** 39.

Krokan H., Haugen A., Myrnes B., and Guddal P. H. (1983) Repair of premutagenic DNA lesions in human foetal tissues: Evidence for low levels of O^6-methylguanine-DNA-methyl transferase and uracil DNA-glycosylase activity in some tissues. *Carcinogenesis* **4,** 1559–1564.

Kuenzle C. C. (1985) Enzymology of DNA-replication and repair in the brain. *Brain Res. Rev.* **10,** 231–245.

Kurtz O. I. and Sinex F. M. (1967) Age related differences in the association of brain DNA and nuclear proteins. *Biochem. Biophys. Acta* **145,** 840–842.

Lai L. W., Ducore J. M., and Rosensteim B. S. (1987) DNA-protein crosslinking in normal human skin fibroblasts exposed to solar ultraviolet wavelengths. *Photochem. Photobiol.* **46,** 143–146.

Lambert M. W., Fenkart D., and Clarke M. (1988) Two DNA endonuclease activities from normal human and xeroderma pigmentosum chromatin active on psoralen plus ultraviolet light treated DNA. *Mutation Res.* **193,** 65–73.

Lehmann A. R., Kirk-Bell S., Ariett C. F., Harcourt S. A., De Weerd-Kastelein E. A., Keijzer W., and Hall-Smith P. (1977) Repair of UV-damage in a variety of human fibroblast cell strains. *Cancer Res.* **37,** 904–910.

Lesko S. A. (1982) DNA-protein and DNA interstrand cross-links induced in isolated chromatin by hydrogen peroxide and ferrous EDTA chelates. *Biochemistry* **21,** 5010–5015.

Lindahl T. (1977) DNA repair enzymes acting on spontaneous lesions in DNA, in *DNA-Repair Processes* (Nichols W. W. and Murphy D. G., eds.), Symposia Specialists, Miami, FL, pp. 225–240.

Lindahl T. (1979) DNA-glycosylases, endonucleases or apurinic/apyrimidinic sites and base excision repair. *Prog. Nucl. Acid Res. Mol. Biol.* **22,** 135–188.

Lindahl T. and Karlstrom O. (1973) Heat induced depyrimidination of deoxyribonucleic acid in neutral solution. *Biochemistry* **12,** 5151–5154.

Lindahl T. and Nyberg B. (1972) Rate of depurination of native DNA. *Biochemistry* **11,** 3610–3618.

Lindahl T. and Nyberg B. (1974) Heat induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry* **13,** 3405–3410.

Linn, S. (1982) Nucleases involved in DNA-repair, in *Nucleases* (Linn S. and Roberts R. J., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 59–83.

Lippke J. A., Gordon L. K., Brash D. E., and Haseltine W. A. (1981) Distribution of UV light induced damage in a defined sequence of human DNA: Detection of alkaline-sensitive lesions at pyrimidine nucleoside cytidine sequences. *Proc. Natl. Acad. Sci. USA* **78,** 3388–3392.

Loeb L. A. and Preston B. D. (1986) Mutagenesis by apurinic/apyrimidinic sites. *Ann. Rev. Genet.* **20,** 201–230.

Madhani H. D., Bohr, V. A., and Hanawalt P. C. (1986) Differential DNA repair in a transcriptionally active and inactive proto-oncogene:c-abl and c-mos., *Cell* **45,** 417–423.

Martin G. M. (1978) Genetic syndromes in man with potential relevance to the pathobiology of aging, in *Genetic Effects on Aging. Birth Defects, Original Article Series* (Bergsma D. and Harrison D. E., eds.), The National Foundation March of Dimes, New York, pp. 5–39.

Massie H. R., Samis H. V., and Baird M. B. (1972) The kinetics of degradation of DNA and RNA by H_2O_2. *Biochem. Biophys. Acta* **272,** 539–548.

Mayne L. V. and Lehmann A. R. (1982) Failure of RNA synthesis to recover after UV irradiation. An early

defect in cells from individuals with cockayne's syndrome and xeroderma pigmentosum. *Cancer Res.* **42,** 1473–1478.

Mayne L. V., Mullenders L. H. F., and Van Zuland, Z. (1988) Cockayne's syndrome: A UV sensitive disorder with defect in the repair of transcribing DNA but normal overall excision repair, in *Mechanisms and Consequences of DNA Damage Processing* (Friedberg E. C. and Hanawalt P. C., eds.), Alan R. Liss, New York, pp. 349–359.

Mckinnon P. J. (1987) *Ataxia Telangiectasia.* An inherited disorder of ionizing radiations sensitivity in man. *Human Genet.* **75,** 197.

Mellon I., Spivak G., and Hanawalt P. C. (1987) Selective removal of transcription blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* **51,** 241–249.

Mellon I. and Hanawalt P. C. 1989) Induction of the *E. coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* **342,** 95–98.

Miller D. L., Reese J. A., and Frazier M. E. (1989) Single strand DNA breaks in human leukocytes induced by ultrasound *in vitro. Ultrasound Med. & Biol.* **15,** 765–771.

Mori N. and Goto S. (1982) Estimation of the single stranded region in the nuclear DNA of mouse tissues during aging with special reference to the brain. *Arch. Gerontol. Geriatr.* **1,** 143–150.

Mullaart E., Lohman, P. H. M., Berends F., and Vijg J. (1990) DNA damage metabolism and aging. *Mutation Res.* **237,** 189–210.

Mullaart E., Boerrigter M. E. T. I., Boer G. J., and Vijg J. (1990) Spontaneous DNA-breaks in the rat brain during development and aging. *Mutation Res.* **237,** 9–15.

Murthy M. R., Barucha A. D., Jacob J., and Roux-Murthy P. K. (1976) Molecular biological models in geriatric neurobiology, in *Neuropsychopharmacology* (Deniker P., Radowco-Thomas C., and Villeneuve A., eds.), Pergamon, Oxford, UK, pp. 1615–1622.

Nakanishi K., Shima A., Fukuda M., and Fujita S. (1979) Age associated increase of single-stranded regions in the DNA of mouse brain and liver cells. *Mech. Aging Dev.* **10,** 273–281.

Niedermuller H. (1985) DNA-repair during aging, in *Molecular Biology of Aging. Gene Stability and Gene Expression* (Sohal R. S., Birnbaum L. S., and Cutler R. G., eds.), Raven, New York, pp. 173–193.

Ono T. and Okada S. (1978) Does the capacity to rejoin radiation induced DNA-breaks decline in senescent mice? *Int. J. Radiat. Biol.* **33,** 403–407.

Ono T., Okada S., and Sugahara T. (1976) Comparative studies of DNA size in various tissues of mice during the aging process. *Exp. Gerontol.* **11,** 127–132.

Peak M. J., Peak J. G., and Jones C. A. (1985) Different (direct & indirect) mechanisms for the induction of DNA-protein crosslinks in human cells by far and near ultraviolet radiations (290 & 405 nm). *Photochem. Photobiol.* **42,** 141–146.

Price A. and Lindahl T. (1991) Enzymatic release of 5'-terminal deoxyribose phosphate residues from damaged DNA in human cells. *Biochemistry* **30,** 8631–8637.

Price G. B., Modak S. P., and Makinodan T. (1971) Age associated changes in the DNA of mouse tissue. *Science* **171,** 917–920.

Rajagopal S. V. and Subba Rao K. (1992) Purification and properties of a deoxyribonuclease (acid DNase) from rat brain. *Biochem. Arch.* **8,** 135–142.

Randerath E., Avitts T. A., Reddy M. V., Miller, R. H., Everson, R. B., and Randerath K. (1986) Comparative ^{32}P-analysis of cigarette smoke induced DNA damage in human tissues and mouse skin. *Cancer Res.* **46,** 5869–5877.

Richter G., Park J. W., and Ames B. N. (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. USA* **85,** 6465–6467.

Rupert C. S. (1975) Enzymatic photoreactivation: Overview, in *Molecular Mechanisms for Repair of DNA* (Hanawalt P. C. and Setlow R. B., eds.), Plenum, New York, pp. 73–87.

Rydberg B. and Lindahl T. (1982) Nonenzymatic methylation of DNA by intracellular methyl group donor S-adenosyl methionine is a potentially mutagenic reaction. *Embo. J.* **1,** 211–216.

Sancar A. and Sancar G. B. (1988) DNA-repair enzymes. *Ann. Rev. Biochem.* **57,** 29–67.

Satoh M. S. and Lindahl T. (1992) Role of poly(ADP-ribose) formation in DNA repair. *Nature* **356,** 356–358.

Saul R. L. and Ames B. N. (1985) Background levels of DNA damage in population, in *Mechanisms of DNA Damage and Repair* (Simic M., Grossman L., and Upton A., eds.), Plenum, New York, pp. 529–536.

Saul R. L., Gee P., and Ames B. N. (1987) Free radicals, DNA-damage and aging, in *Modern Biological*

Theories of Aging (Warner H. R., Butler R. N., Sprott R. L., and Schneider, E. L., eds.), Raven, New York, pp. 113–129.

Schapper R. M. and Loeb L. A. (1981) Depurination causes mutations in SOS-induced cells. *Proc. Natl. Acad. Sci. USA* **78,** 1773–1777.

Schmickel R. D., Chu E. H. Y., Trosko J. E., and Chang C. C. (1977) Cockayne syndrome: A cellular sensitivity to ultraviolet light. *Pediatrics* **60,** 135–139.

Shibutani S., Takeshita M., and Grollmann A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation. Damaged Base 8-oxod G. Nature **349,** 431–434.

Sedgwick S. G. (1986) Stability and change through DNA-repair, in *Accuracy in Molecular Processes: Its Control and Relevance to Living Systems* (Kirkwood T. B. L., Rosenberger R. F., and Galas D. S., eds.), Chapan and Hall, London, pp. 233–289.

Su C. M., Brash D. E., Turturro A., and Hart R. W. (1984) Longevity dependent organ specific accumulation of DNA-damage in the closely related urine species. *Mech. Aging Dev.* **27,** 239–247.

Subba Rao K. (1990) DNA-repair in developing and aging brain. *Proc. Indian Natl. Sci. Acad.* **B56,** 141–150.

Subba Rao K. V. and Subba Rao K. (1982) Changes in DNA, RNA, protein and the activities of acid and alkaline DNases in grey and white matter regions of aging rat brain. *Mech. Aging Dev.* **18,** 225–238.

Subba Rao K. V. and Subba Rao K. (1984) Increased DNA-polymerase β activity in different regions of aging rat brain. *Biochem. Intl.* **9,** 391–397.

Subba Rao K., Martin G. M., and Loeb L. A. (1985) Fidelity of DNA-polymerase-β in neurons from young and very aged mice. *J. Neurochem.* **45,** 1273–1278.

Subba Rao K., Venugopal J., and Ramesh Kumar P. (1992) Alterations in chromatin structure and transcriptional activity in aging rat brain, in *Recent Advances in Physiological Sciences* (Manchanda S. K., ed.), Macmillan India Limited, New Delhi, pp. 425–431.

Subrahmanyam K. and Subba Rao K. (1991) Ultraviolet light induced unscheduled DNA-synthesis in isolated neurons of rat brain of different ages. *Mech. Aging Dev.* **57,** 283–291.

Sung S. C. (1968) Deoxyribonucleases from rat brain. *J. Neurochem.* **15,** 477–481.

Szilard L. (1959) On the nature of the aging process. *Proc. Natl. Acad. Sci. USA* **45,** 30–45.

Tan B. H., Bencsath F. A., and Gaubatz J. W. (1990) Steady state levels of 7-methylguanine increase in nuclear DNA of postmitotic mouse tissues during aging. *Mutation Res.* **237,** 229–238.

Thompson, L. H. (1989) Somatic cell genetics approach to dissecting mammalian DNA repair. *Environ. Mol. Mutagen.* **14,** 264–281.

Tobin A. J. and Khrestchatisky M. (1989) Gene expression in the mammalian nervous system, in *Basic Neurochemistry,* 4th ed. (Siegel G., Agranoff B., Albers R. W., and Molinoff P., eds.), Raven, New York, pp. 417–428.

Venugopal J. and Subba Rao K. (1991) Gene expression in different cell types of aging rat brain. *J. Neurochem.* **56,** 812–817.

Vos J. M. and Hanawalt P. C. (1987) Processing of psoralen adducts in active gene: Repair and replication of DNA containing monoadducts and interstrand crosslinks. *Cell* **50,** 1789–1799.

Warner H. R. and Price A. R. (1989) Involvement of DNA-repair in cancer and aging. J. *Geront.* **44,** 45–54.

Waser J., Hubscher U., Kuenzle C. C., and Spadari S. (1979) DNA polymerase β from brain neurons is a repair enzyme. *Eur. J. Biochem.* **97,** 361–368.

Washington W. J., Foote R. S., Dunn W. C., Generoso W. M., and Mitra S. (1989) Age dependent modulation of tissue-specific repair activity or 3-methyladenine and O^6-methylguanine in DNA of inbred mice. *Mech. Aging Dev.* **48,** 43–52.

Watson J. D. and Crock F. H. C. (1953) Molecular Structure of Nucleic Acids: A structure for Deoxyribose Nucleic Acid. *Nature,* **177,** 737.

Wheeler K. T. and Lett J. T. (1974) On the possibility that DNA-repair is related to age in non-dividing cells. *Proc. Natl. Acad. Sci. USA* **71,** 1862–1865.

Wiestler O., Kleihues P., and Pegg A. E. (1984) O^6-Alkylguanine-DNA-alkyltransferase activity in human brain and brain tumors. *Carcinogenesis* **5,** 121–124.

Yamane T., Wylnda B. J., and Shulman R. G. (1967) Dihydrothymine from UV irradiated DNA. *Proc. Natl. Acad. Sci. USA* **58,** 439.