CHAPTER 1

INTRODUCTION

INTRODUCTION

Aging can be defined as the time related deterioration of physiological functions necessary for survival and fertility. Although usually assumed to start after maturity, most of the signs of the aging are not obvious until later in life. Aging changes are manifest at all levels of organization -molecular to organismic level and changes occurring after attaining reproductive maturity comprise the phenomenon of aging or senescence. However, fundamental molecular mechanisms involved in aging remains controversial and largely unproven and the major reason for this is the obvious complexity of the problem.

The average life span of humans has increased dramatically over the time, yet the maximum life span potential has remained approximately constant and is usually stated to be 90-100 years (Cutler, 1990). Benjamin Gompertz (1825) in the early 19th century first described an exponential increase in mortality with aging due to various causes, a phenomenon that is still seen today (Gompertz, 1825). The rapid advances in the medicine and biology has enabled control of infectious diseases responsible for the majority of deaths. Due to this progress in medicine and health care practice, average life span of humans has been on the increase during the past 100 years.

Powerful tools of molecular biology are now being applied by scientists to evaluate the leading hypotheses explaining aging process. This kind of work provides the scientific foundation to enhance the quality of life for people suffering the failings of age (Cristofalo, 1994)

First evolutionary arguments to explain the phenomenon of aging was given by August Weismann (1834–1914), the great German theorist and experimental biologist of the 19th century. His initial idea was that there exists a specific death-mechanism designed by natural selection to eliminate the old and worn-out members of a population. The purpose of

this programmed death of the old is to clean up the living space and to free up resources for younger generations.

THEORIES OF AGING

Many theories about the cause(s) of aging have been proposed over the years in an effort to adequately explain the phenotype of aged organisms. Modern technology and research techniques have allowed the researchers to test many of these theories of aging. Some theories have been proven to be related to aging where as others show high probability or possibility of affecting the aging, while others have been disproven. No single theory explains the various biochemical changes occurring during the process (Rattan and Clark, 1988; Gensler and Bernstein, 1981). In some cases, emphasis is given to some of the genetic factors as determinants of the process, while others give importance to random accumulation of damage such as that caused by mutations, errors, free radicals, in addition to genetic component (Smith, 1962; Warner, 1987; Rattan and Clark, 1988; Finch, 1990; Bernstein and Bernstein, 1991; Rao, 1993; Kanungo, 1994).

Every single hypothesis formulated on scientific basis, has its validity. There are programmed processes that contribute to the process of aging, and of course, the errors occurring during the lifetime of a cell speed up the process of aging.

Attempts have also been made to divide various theories of aging into two or three categories. Today such characterization has lost it's credibility since many of the theories, therefore the categories are not mutually exclusive. Only the emphasis on a particular aspect might vary. Therefore, a brief description of various theories/concepts/hypotheses to explain the possible mechanisms of aging phenomenon, is given below without any regard to the characterization since many of these concepts overlap with each other.

SOMATIC MUTATION THEORY

Somatic mutation theory is the most prominent among the theories that lay emphasis on stochastic factors and was proposed by Szilard (1959) which states that the accumulation of mutations during aging would result in decreased protein function thereby compromising the ability of cells to perform their respective function and ultimately to cell death. This theory arose from the observations that a sublethal dose of radiation shortens the lifespan of mammals.

Clark and Rubin, (1961) *in vivo* and Hoehn et al., (1975) *in vitro* showed that the effects of ionizing radiation on life shortening may be non specific and may occur due to radiation syndrome which is unrelated to natural aging. Post mitotic cell suffers from the validity of this theory as there is no means of measuring the range of mutations in these cells and the only way it has been measured is evaluating the mortality rate, which may be due to several other factors. The drawbacks of somatic mutation theory is that it cannot explain the ·nsitivity of germ cells of long lived species such as humans to ionizing radiation than those of mice and drosophila. Sinex, (1974), Strehler, (1964) and Walburg et al., (1966) have shown in Drosophila and mice respectively that acceleration of aging after exposure to ionizing radiation may be due to secondary effects, although at higher doses, the effects of mortality are evident.

ERROR CATASTROPHE

The error catastrophe theory proposes that random errors occur eventually in protein synthesizing machinery, that synthesize DNA or other template molecules and was proposed first by Orgel (1963). Errors occurring in proteins are lost by natural turnover and simply replaced with error free molecules. Errors in protein synthesizing machinery that introduce errors in molecules could result in rapid accumulation of error containing

molecules that would result in "error catastrophe" that would be incompatible with normal function and life. Studies by Linn et al., (1976); Murray and Holliday (1981); Krauss and Linn, (1982) have shown that various DNA Polymerases isolated from fibroblasts aging in culture are less faithful in copying synthetic polynucleotides than the polymerases isolated from early passage cells.

Contrary to this occurrence of errors in proteins that may cause aging, there are studies showing no changes in the primary structure of enzymes as a function of age (Kanungo and Gandhi, 1972; Patnaik and Kanungo, 1976; Srivastava, 1971). Although there are numerous reports of altered proteins in aging, no direct evidence of age dependent protein mis-synthesis has yet been reported. The altered proteins that occur in aging cells and tissues are supposed to be due to post-translational modifications such as oxidation and glycation (Kristal, 1992; Levin and Stadtman, 1996). The increase in altered proteins appear to be due to decreased clearance in older cells (Gracy et al., 1985).

CODON RESTRICTION THEORY

Fidelity and/or accuracy of mRNA message translation is impaired with aging due to cells inability to decode the triplet codons (bases) in mRNA molecules (Strehler et al., 1971). Degenerative tRNAs are the decoding molecules of the genetic code and the qualitative changes in the iso-acceptor tRNAs of amino acids may alter the rate of decoding of the message and this in turn affects translation.

This theory however does not explain the factors responsible for altering the gene expression for tRNAs, sythetases and those responsible for the post-translational modifications of tRNAs. The fundamental cause of aging remains elusive, as these changes are secondary in nature.

FREE RADICAL THEORY

The Free radical theory was initially proposed by Harman (1956,1981) which says that most aging changes are due to damage to the molecules by free radicals, which are atoms or molecules that contain an unpaired electron and are therefore highly reactive. Accumulation of age pigment-Lipofuscin with age due to damage directly affecting the Lipofuscin (Miquel et al., 1977) is often cited as supporting example for free radical theory of aging.

The production of free radicals is an unavoidable consequence of aerobic metabolism and the prominent members of Reactive oxygen species (ROS) metabolism such as catalase (CAT) and superoxide dismutase (SOD) protect cells against hydrogen peroxide and superoxide radicals respectively. ROS cause critical damage to chromosomes, resulting in mutations and translation errors, which is the basis for many cancers. Since most cancers are age related, it is suggested that relationship between cancer and ROS involve free radicals.

A direct test for free radical theory of aging would be extension of lifespan upon administration of antioxidants. Although a number of studies have been done, the results on balance are ambiguous. Comfort (1979); Halliwell and Gutterridge (1989) have shown that extension of average life span by antioxidants is minimal or absent in mammals, where as it has been demonstrated in lower organisms (Drosophila, nematodes, rotifers). Possibility of attempting to prolong life span by administrating antioxidants to complex organisms may fail as they are unable to access the area of ROS damage even in sufficient concentration. In general DNA damage hypothesis and free radical theory are compatible in that the most important target of free radical damage is DNA, and that other oxidatively damaged molecules are efficiently replaced as long as DNA is intact.

GENE REGULATION THEORY

According to "gene regulation theory" of aging, the depletion of certain "nuclear proteins or factors" responsible for keeping essential genes active leads to depressed functions of organs. Differentiation and growth occur by sequential activation and repression of certain genes unique to these phases. The products and byproducts of these genes after reaching critical levels and at specific time stimulate certain unique genes responsible for reproductive phase. Triggering of expression of these genes occurs by some product of genes (like hormones and other factors) that confer reproductive ability to organism. Failure to maintain a balance of factors and modulators results in the loss of reproductive ability and other functions. Thus decline in physiological functions may begin and lead to senescence (Kanungo, 1980).

NEUROENDOCRINE THEORY

Finch (1972) proposed that those functional decrements in neurons and their associated hormones are central to aging process. (McGeer and McGeer, 1975; Finch, 1978) evidenced a decrease in brain catacholamines and the reduction in catecholamine receptors in the corpus striatum and cerebellum of old rats (Greenberg and Weiss, 1978). Denckla (1975) showed that hypophysectomy in rodents followed by replacement of known hormones maintains or may extend lifespan. In addition, reductions in brain dopaminergic neurotransmission are more prominent in a short-lived rat strain (Cotzias et al., 1977). The mechanisms responsible for hormone receptor alterations during aging are unknown. Accumulation of DNA damage in non-dividing cells is likely to lead to reduced/altered gene expression. This may explain both the decreased population of hormone receptors and decline in general neuroendocrine function. This neuroendocrine theory of aging is compatible with the DNA damage hypothesis of aging.

IMMUNOLOGICAL THEORY

Walford (1969) proposed immunological theory based upon two main observations. First the functional capacity of the immune system declines with age, as evidenced by a decreased response of T cells to mitogens and reduced resistance to infectious diseases and increase in autoimmune phenomenon with age, such as increase in serum autoantibodies.

Organisms that share aspects of aging with higher organisms lack a complex immune system. This theory fails to interpret the aspects which distinguish between the immune system and the fundamental changes occurring in many types of cells and tissues and also the secondary effects mediated by aging altered immune system (Troen, 2003).

PROTEIN MODIFICATION

Changes in function are seen in addition to the changes in the steady state level of proteins with age. Aging is accompanied by decreased specific activity in many enzymes, altered heat stability, and increased carbonyl content of proteins (Levin and Stadtman, 1996). Kohn (1978) and Bjorkstein (1974) hypothesized that the accumulation of post translationally altered proteins could impair cellular, and ultimately, organ functions.

Some of such alterations could lead to increased functions at some sites and impaired functions at other sites the example being increased collagen crosslinking with age (Reiser et al., 1987). The cross-linking of macromolecules such as collagen, elastin, osteocalcin, and the eye lens protein crystallin could alter both the extracellular matrix and organ function. These covalent protein-protein interactions probably play a role in increased stiffness of vascular walls with aging (Troen, 2003).

TELOMERES AND AGING

During the past few years a new concept has emerged which again adds credibility to the theory of DNA damage and repair in explaining aging as well as cell replication and transformation of somatic cells into malignant cells. This hypothesis is based on results suggesting the non coding telomeric DNA (located at the tips of the eukaryotic chromosomes) may have a telling role in DNA replication (cell replication) and therefore in the phenomenon of cancer and aging. Telomeres are the physical ends of the chromosomes, which in mammals are composed of tandem repeats of TTAGGG (Meyne et al., 1989) and appear to stabilize the structure of chromosomes. Apart from providing stability to chromosomes, telomeres carry out another crucial function in replicating cells-the ability to allow the end of the linear DNA to be replicated completely without the loss of terminal bases at 5'- end. Such loss is predicted as a natural consequence derived from the properties of the replicative machinery of conventional semiconservative replication (Olovnikov, 1973). Von Zglinciki, (2000) suggested that the most important factor leading to the shortening of telomeres as the oxidative damage. The lost sequences of the telomere at each round of replication are synthesized again by the enzyme telomerase, a ribonucleoprotein (Blackburn, 1992).

Many of the cells in an adult animal are either quiescent or post mitotic and they proliferate rarely or not at all and their telomeres might not shorten significantly during the life of an individual. It is interesting that in certain human syndromes, characterized by features of premature aging viz., Progeria and Werner's syndrome, the average telomeric lengths are significantly shorter than in normal individuals, thus pointing out a relationship between average telomeric length and aging (Lansdorp, 2000).

DNA DAMAGE AND REPAIR THEORY

Alexander (1967) was the first to suggest that DNA damage per se, apart from its role in inducing mutations, may be primary cause of aging. This theory postulates that the DNA damage, which is bound to occur in the body of an organism, is repaired efficiently upto certain age of an organism but thereafter it is compromised in a predetermined manner. Thus, from some point of lifespan DNA repair capacity decreases, therefore DNA damage accumulates. This accumulation of DNA damage leads to the breakdown of all the vital process in the cell finally leading to the death.

Hart and Setlow (1974) observed a direct relationship between maximum achievable lifespan of a species and its capacity for UV induced unscheduled DNA synthesis (UDS) [a measure of DNA repair capacity] in fibroblast from seven species. Similar observations were made using fibroblast from primates (Hart and Daniel, 1980) between two mouse species with a difference in Lifespan of 2.5 fold (Hart et al., 1979b), in skin cells of humans (Sutherland et al., 1980) and in lens epithelial cells from rat, rabbit, dog, cow, horse (Treton and Courtois, 1982).

Wei et al., (1993) demonstrated in basal cell carcinoma skin cancer patients that the normal decline in DNA repair with increased age may account for the increased risk of skin cancer that begins in middle age, suggesting that the occurrence of skin cancer in the young may represent precocious aging.

Cortopassi and Wang, 1996 demonstrated that the rate of mitochondrial mutagenesis in laboratory mouse is exponential and is 40 times faster than humans, which is in consistent with the lifespan of mice. Zahn et al.,(2000) showed in two mouse strains that the strain with shorter longevity, the damage increases and the repair deficiencies are drastically

deviating from those with higher longevity. These findings of strong coupling of the DNA status to aging as well as longevity suggest causative relations.

De Boer et al., (2002) showed in mice with a mutation in XPD, a gene encoding a DNA helicase that is mutated in the human disorder trichothiodystrophy (TTD) that aging in TTD mice is caused by unrepaired DNA damage that compromises transcription, leading to functional inactivation of critical genes and enhanced Apoptosis.

There are also a few studies that do not support DNA damage contributing to aging. Studies *in vitro* of senescent fibroblast showed minimal decrease in DNA repair (Hart and Setlow, 1976). Similarly, another study showed that the capacity to repair DNA Single and Double strand breaks mediated by ionizing radiation is not altered during *in vitro* cellular senescence (Mayer et al., 1989).

However there is extensive correlative evidence that DNA damage and mutations increase with age. In addition, there are studies that have demonstrated a corresponding decrease of DNA repair. This decrease in DNA repair may in part account for the increased DNA damage levels and mutation frequencies observed with age.

In mammals, long-lived neurons, differentiated muscle cells, and other differentiated cell types that do not divide or divide only slowly, accumulate DNA damage with age. These cells are likely candidates to govern the rate of mammalian aging. In brain the level of DNA repair is low, endogenous damages accumulate with age, mRNA synthesis declines, and protein synthesis is reduced. Furthermore, cell loss occurs, tissue function declines, and functional impairments directly related to the central processes of aging occur. Thus, for the brain, there appears to be a direct relationship between the accumulation of DNA damage and the important feature of aging. In contrast to non-dividing or slowly dividing cells cell populations, atleast some types of rapidly dividing cell populations

appear to cope with DNA damage by replacing lethally damaged cells through replication of undamaged ones. Examples include duodenum and colon epithelial cells and hemopoitic cells of bone marrow (Bernstein and Bernstein, 1991).

It is opinion of this lab that DNA-damage and repair theory occupies a central role in explaining the mechanisms of aging phenomenon at a basic and fundamental level. This concept has the necessary depth to compliment many other theories of aging either partly or fully. Moreover, the work presented in this thesis pertains to the DNA-repair capacity of brain cells during aging. In view of this, an attempt is made below to briefly review the existing knowledge about the DNA-damage and DNA-repair in aging tissues with a special emphasis on brain.

DNA DAMAGE

Living cells face the tremendous task of maintaining an intact genome during the lifespan. The genetic information of all organisms and many viruses is stored in the form of stable molecule DNA. Since loss of DNA signifies loss of genetic information, DNA has to be maintained. This is in contrast to other biological macromolecules, which can be degraded and replaced by newly synthesized molecules. DNA repair and replication are flanked by a continuous surveillance of genome integrity. When DNA damage or a replication block is detected, checkpoints are activated that delay cell cycle progression. At the same time, DNA repair genes and other factors are activated to remove the damage or replication block, or, in case the DNA damage is too extensive, to initiate programmed cell death. In this way, premature progression into the next phase of the cell cycle is prevented, and changes in the genetic material in the form of heritable mutations is obviated. The nature of the genetic component involved in aging is complex. Several possible mechanisms have been identified which may contribute to the aging process. The most obvious change is

seen in gene expression of altered forms of proteins or altered levels of particular proteins. Alterations in the integrity of DNA itself could contribute to the aging process. Many thousands of mutations may occur in each cell per day as a result of oxidative damage (Lindahl, 1993). Though the DNA remains intact to a large extent during the life of an animal, the efficiency of the DNA repair machinery may decline with age (Bohr and Anson, 1995; Walter et al., 1997).

There are observations supporting that DNA repair may be more efficient in cells from longer lived species (Burkle et al., 1992; Grube and Burkle, 1992).

A plethora of alterations in the native structure of DNA occurs in the cell both due to external and internal factors. In view of the highly protective nature of the brain (including the blood brain barrier), the major enemy for causing DNA damage is only from within the brain. The net rate of accumulation of a particular type of DNA damage depends on both the rate of its occurrence and the rate of its removal by repair enzymes (Hart and Setlow, 1974).

DNA DAMAGES BY ENDOGENOUS FACTORS

AP- Sites

Apurinic/apyrimidinic damages can occur under physiological conditions by hydrolytic cleavage of the purines/pyrimidines from the deoxyribose phosphate backbone of DNA. It is estimated that a mammalian cell at 37^0C loses about 10,000 purines and 500 pyrimidines from its DNA by spontaneous hydrolysis (Lindhal, 1977) and it should be promptly removed from the DNA as it is a non-coding lesion that can lead to misincorporation during replication and transcription (Friedberg et al., 1995). The amount of DNA depurination caused by non enzymatic (spontaneous) hydrolysis that occurs in a single long lived, non replicating mammalian cell, such as human neuron, was estimated to be about 10^8 purine bases during the lifespan. This accounts to about 3% of total number of

purines in the cell's DNA (Lindahl and Nyberg, 1972). Thus, DNA is significantly unstable at the temperatures at which mammalian cells normally exist.

Mismatches and altered bases

Normal metabolic reactions may affect spontaneous deamination of bases in DNA. The products of deamination are mutagenic and would therefore interfere with correct transcriptional process in brain. The deamination of cytosine to uracil is one of the ways by which uracil, a base in RNA, can occur in DNA. Bases in the DNA can also be modified through alkylation in a non enzymatic way by compounds like S-Adenosylmethionine that leads to the formation of N -methylguanine, N -methyladenine and O -methylguanine (Barrows and Magee, 1982; Rydberg and Lindahl, 1982). The methylated bases are eventually converted to strand break.

Oxidative damage to the bases in cellular DNA can be caused by products of oxidative metabolism like superoxide radical (O_2^-), hydroxyl radical (OH), Hydrogen peroxide (H2Q2).

DNA DAMAGE BY EXOGENOUS FACTORS

Dimers of Pyrimidines

Dimerized pyrimidines are very stable at extreme pressures and temperatures and pose a real threat to genomic integrity. UV light of wavelength around 260 nm induces the formation of chemical bonds between adjacent pyrimidines in DNA and form pyrimidine dimers. Tice and Setlow (1985) estimated that the rate at which UV irradiation induces pyrimidine dimers in human skin is 50,000 per cell per hour. Exposure to both near and far UV light forms several photoproducts (Rao, 1993). Damage from UV light to the brain is quite limited since the brain is very well protected by skull. Even so UV induced damage is routinely used as model system with various tissues including brain (Rao, 1997).

Single strand breaks (SSB)

Single strand breaks are the most prevalent DNA-damage in mammalian cells. Single strand breaks may be formed from AP sites at alkaline pH, removal of modified base by suitable glycosylase in the initial step of Base excision repair. UV and ionizing radiations can cause SSB's by generation of free radicals directly or indirectly (Mullart et al., 1990). Single strand breaks could be a good marker for the DNA damage status in any cell.

Double strand damages: Cross-links and Double strand breaks(DSB)

Ultraviolet light, X-ray and gamma ray irradiation is known to induce cross-links, DSB and SSB. Important class of chemical modification in DNA is interstrand cross-links since they prevent strand separation needed for replication and also transcriptional process. About eight or nine interstrand cross links occur in each mammalian cell per day (Bernstein and Bernstein, 1991).

It can be assumed that in view of the protective situation of brain and due to its high metabolic activity the major damage to the DNA would emanate from the endogenous factors and from such exogenous factors that can cross the blood brain barrier.

The frequency of occurrence of different DNA damages by various factors is summarized in the Table I.

TABLE 1: Estimated Rates of Occurrence of Endogenous DNA Damages in Mammalian Cells

Damage	Events per Day/cell	Reference
Depurination	12,000 13,920	Lindahl, 1977 Tice and Setlow, 1985
Depyrimidination	600 696	Lindahl, 1977 Tice and Setlow, 1985
Deamination	100-300	Lindhal and Nyberg, 1974 Tice and Setlow, 1985
Single-strand breaks (Including all types of Base damage Viz.. Oxidative damage, Adduct formation with reducing sugars, methyalation, Cross-links, a d so forth)	20,00-40,000	Saul and Ames, 1985
Double-strand break	8.8	Bernstein and Bernstein, 1991
Interstrand cross-link	8.0	Bernstein and Bernstein, 1991
DNA-protein cross-link	unknown	Bernstein and Bernstein, 1991

Bernstein C and Bernstein H (1981) "Aging, Sex, and DNA repair" Academic press Inc. San Diego, California.

DNA DAMAGE IN BRAIN

There are some studies to look into DNA damage in brain and these studies were conducted to measure the accumulation of DNA damage with respect to age. Most of these studies appear to check the validity of a number of aging theories that have the 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). Price et al., (1971) showed in mice that accumulation of SSB is more in brain compared to liver with age. Chetsanga et al., (1977) reported that alkaline sucrose gradient sedimentation of DNA of mouse brain showed few bands for the old (30 months) and only one for the young (6 months), indicating degradation of DNA in old age owing to the breaks. Murthy et al., (1976) observed more single strand regions in preparations with both isolated chromatin and DNA obtained from old cerebral cortex as compared to those from young. Mori and Goto (1982) using single strand specific S1 endonuclease assay showed that younger mice brain DNA contained only 2.0% single strand regions than mice aged 30 months. Interestingly they could not find any such age associated changes in other organs like liver, kidney, heart, and spleen.

Tan et al., (1990) showed that steady state level of 7-methylguanine and major product formed by methylating agents both *in vitro* and *in vivo* went up approximately 2 fold between young and old age. Mandavilli and Rao, (1996) showed that the number of SSBs increase with age in both the cell types and in all the regions studied viz., cerebral cortex, cerebellum, hippocampus, hypothalamus and brain stem. Highest number of SSBs were 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.

In contrast to the above findings, there are a small number of studies that reported no age dependent increase of DNA damage in brain. Ono et al., 1976; Su et al., 1984; Mullart et al., 1990). The reasons for these discrepancies are not clear as of now.

Alterations with age at the genetic level were also observed by some workers. Studies by Kanungo and Thakur (1979); Chaturvedi and Kanungo (1985) showed enhanced condensation or compaction of chromatin with age in rat brain. Their results also showed a 50% reduction in the RNA-Polymerase II activity in rat brain in old age which may be a result of structural changes in chromatin that may occur with increasing age. Studies using enzyme monococcal nuclease as a probe for chromatin structure Berkowitz et al., (1983) observed that DNA from neuronal preparations showed a decreased susceptibility to digestion during aging. They also observed dramatic increase in the nucleosome spacing of the chromatin. All the above studies with the overwhelming literature point that with the advancement of age there is accumulation of DNA damage in brain.

DNA REPAIR

As a major defense against the environmental damage to cells, DNA-repair is present in almost all the organisms including bacteria, yeast, fish, amphibians, rodents and humans. DNA repair process would minimize cell killing, mutations, persistent DNA damage and errors in replication.

All organisms have therefore developed mechanisms to maintain the integrity of their genome by either preventing damage to DNA or correcting the damage once occurred. The variety of DNA lesions is matched by a multiplicity of avoidance and repair pathways (Eisen and Hanawalt, 1999; Wood et al., 2001). Although the number of gene products that are involved in DNA repair is large in many organisms (more than 100 genes), nature

makes use of a rather limited number of protein domains for DNA repair processes (Aravind and Koonin, 1999; Wood et al., 2001).

The pathways involved in repair of DNA damage in eukaryotic cells were initially categorized using damage sensitive mutants of yeast *Saccharomyces cerevisiae.* More recent characterization of repair has been carried out in metazoans exploiting human genetic repair diseases, mutations in mice, mutations in mammalian cell lines and *in vitro* repair systems (Friedberg et al., 1991; Friedberg et al., 1995) There are three major DNA repair pathways to counteract the different types of DNA damages. 1) A simple reversal of damage 2) Recombinant repair including the endjoining 3) Excision repair including mismatch repair.

1) A simple reversal of damage

Direct reversal of the damage is a simple and important way of dealing with certain DNA lesions. Examples for this mechanism are the removal of alkyl groups by the ubiquitous enzyme alkyltransferase, reversal of the UV-induced pyrimidine dimer formation by the enzyme photolyase, or direct ligation of DNA single strand breaks (Friedberg et al., 1995; Eisen and Hanawalt, 1999). Reversal of damage can take place by a single enzyme O^6-methyl guanine methyltransferase which removes methyl groups from O^6 -methyl guanine thus avoiding the mismatch formation since O^6-methyl guanine can pair with both C or T (Mitra and Kaina, 1993). In these modes of repair there is no cleavage of DNA strand but simply structural alterations are reversed *in situ* .

2) Recombinant type of repair

Both DNA double strand breaks and interstrand cross-links are unusual lesions since they alter both strands of the DNA molecule (Thompson and Schild, 1999). If left unrepaired, DSBs lead to broken chromosomes and cell death, and if repaired incorrectly,

they can lead to chromosome rearrangements and cancer (Chu, 1997). Recombination can occur either by homologous recombination repair (HRR) or non-homologous end joining (NHEJ) the latter mode being less accurate.

3) Excision repair including mismatch repair

Excision repair pathway is the most predominant and perhaps universal one to maintain the genomic integrity. Essentially, the overall strategy in this pathway consists of 4 steps. 1). Recognition of the damage site 2). Excision of the damaged portion. 3). Resynthesis of the removed sequence by DNA Polymerases 4). Ligation of the newly synthesized strand by Ligases.

The overall excision repair constitutes 2 major subpathways-nucleotide excision repair (NER) and base excision repair (BER). The mismatch repair is generally considered as a part of NER.

Nucleotide excision repair (NER)

NER is a highly sophisticated and versatile DNA damage removal pathway. NER removes predominantly bulky DNA adducts and damage that distorts the DNA structure considerably. NER is able to cope with a multitude of DNA lesions, the most relevant of which may be the damage inflicted on DNA by the UV component of sunlight (de Laat et al., 1999). Examples for NER are damage due to exposure to UV irradiation, adduct formation with a variety of compounds like cisplatin, psolaren, carcinogens like acetlyaminoflourine etc. Mechanisms of many of the steps of NER in eukaryotic cells is less known. The available evidence suggests that the overall process resembles that in *E. coli*, but there are many differences in detail.

NER differs from the BER in that excision patch is quite long in NER when compared to shorter patch in BER. 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 and Wood, 1994). It is for this reason, the NER is considered as 'long patch repair' while the BER is routinely considered as 'short patch repair'.

DNA mismatch repair (MMR) plays a significant postreplicative role in safeguarding the integrity of the genome virtually in all organisms from bacteria to mammals. This repair pathway corrects base-base and insertion/deletion (I/D) mismatches that have escaped the proofreading function of replicative polymerases. The human and the bacterial DNA MMR systems are very similar not only in structure, but also in function. Both confer the genome a 100–1000 fold protection against mutations arising during DNA replication (Loeb, 1994), and both systems scan and repair newly replicated DNA by excising the mutated strand in either direction to the mismatch. In its absence, cells assume a mutator phenotype in which the rate of spontaneous mutation is greatly elevated. The discovery that defects in mismatch repair segregate with certain cancer predisposition syndromes hig. ights its essential role in mutation avoidance. Mutations in one of the human DNA MMR genes, hMSH-2, account for approximately half of all cases of genetically linked hereditary non-Polyposis colorectal cancer (Hemminki et al., 1994; Fishel et al., 1993), and inactivation of the mouse MSH2 gene results in a lymphoproliferative disorder and a predisposition to malignancy (de Wind et al., 1995). The human system has a number of homologues for each bacterial protein. The human MMR system may be regulated in several different biological situations. Studies with immunohistochemistry showed that the hMSH2 protein in proliferative portions of oesophageal and intestinal epithelium (Leach et al., 1996; Wilson et al., 1995; Marra et al., 1996) and increases atleast 12 fold in proliferating cells (Marra et al., 1996).

Base excision repair (BER)

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 upto 13 nucleotides (Fig 1). Step one of short patch pathway (left panel of Fig 1) consists of the recognition and cleavage of the altered base (A) from the deoxyribose phosphate moiety by an appropriate DNA-glycosylase. This enzyme also allows the AP endonuclease (APE1) to reach the site. (Fig. 1.1). Multiple DNA glycosylases with varying substrate specificity are continuously scanning the DNA. For example, eight human nuclear glycosylases have been cloned to date (Scharer and Jiricny, 2001). Some DNA-glycosylases recognize and remove 8-oxy guanine opposite C (Rosenquist et al., 1997; Radicella et al., 1997), oxidative forms of bases like thymine glycol, cytosine glycol, dihydrouracil (Hilbert et al.,1997) and alkylated adenine like 3-methyl adenine, ethenoadenine and hypoxanthine (Chakravarthi et al, 1991; Samson et al., 1991).

In the second step (Fig 1.2) DNA chain at 5'-side of the abasic site is cleaved by a major endonuclease APE1 specific for abasic site. APE1 is the major endonuclease in humans, also known as HAP1, APEX, REF1 (Demple et al., 1991; Seki et al., 1992; Robson et al., 1992). 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 p, which is involved in the next step in the repair pathway. The glycosylase dissociates from DNA at this point.

In third step, the Pol β fills up the one-nucleotide gap and also releases the 5'-2-deoxyribose-5- phosphate (dRp). At the same time DNA-ligase III - XRCC1 (X-ray repair cross complementing, gene I) complex arrives at the site.

In the fourth step DNA-ligase III seals the nick and Pol β dissociates from the site. Subsequently the XRCCl and ligase III come off from the site leaving behind repaired DNA (Fig 1 left panel, B).

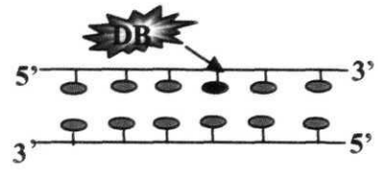
Figure 1

An outline of Pathways of Base Excision Repair (BER): On the left side is "Short patch" or Single nucleotide pathway and on the right side "Long patch" pathway

SHORT PATCH REPAIR

LONG PATCH REPAIR

A.



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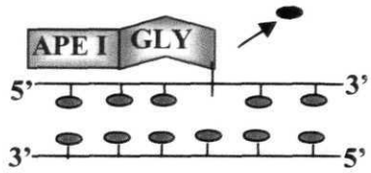

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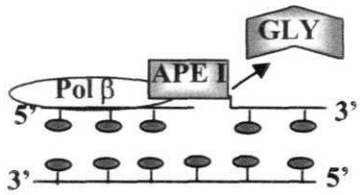

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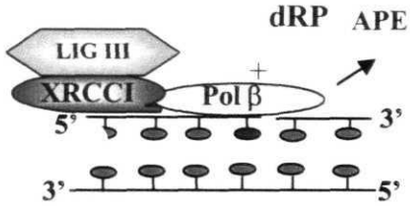

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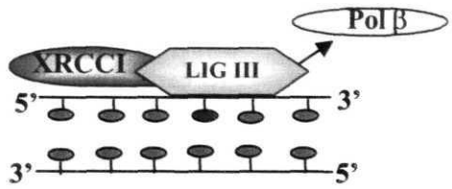

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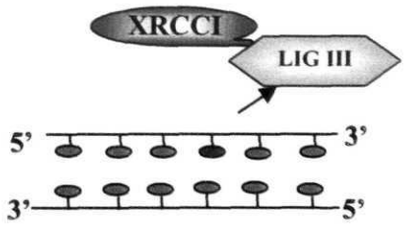

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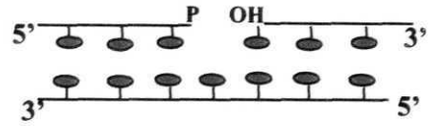

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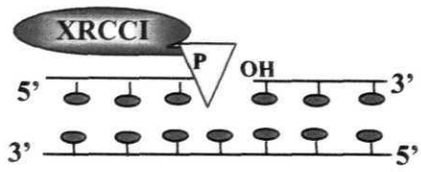

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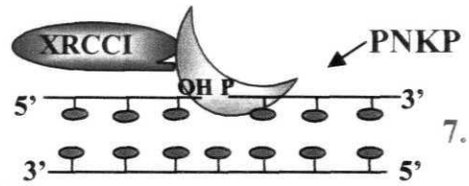

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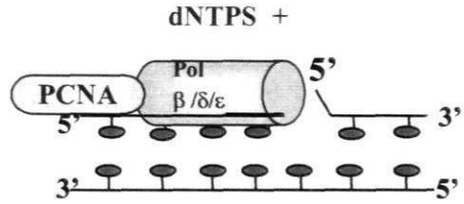

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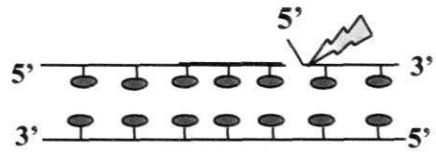

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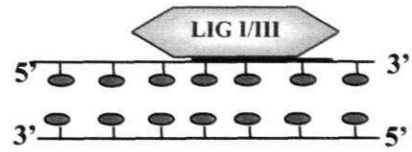

Human XRCC 1 not only complexes with DNA-ligase III but also interacts with other core enzymes involved in BER and is therefore considered to play a crucial role in protein exchanges in the pathway. Eukaryotes, in contrast to prokaryotes, contain more than one DNA ligase, and these enzymes have distinct 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.

The predominant route for BER is the 'short patch or single nucleotide pathway' shown on the left side of Fig 1 .The overall process of BER pathway is characterized by the sequential binding of proteins to DNA as well as among themselves in pairs facilitating the repair process to occur efficiently and swiftly (Kohler et al, 1999; Hoeijmakers, 2001)

When dRpase(5'-2-deoxyribose-5-phosphate lyase) activity of Pol β cannot act on the complex structure of the terminal sugar phosphate after the AP endonuclease incision (Step 2) (for example, reduced or oxidized abasic site) the repair synthesis would nevertheless continue but in a strand displacement manner. According to recent reports, Pol β also initiates regular long-patch BER, which involves synthesis of upto 13 nucleotides beginning at the damage site (Frosina et al., 1996; Klungland and Lindahl, 1997; Stucki et al., 1998; Matsumoto et al., 1999; Dantzer et al., 2000; Podlutsky et al., 2001; Prasad et al., 2001). Poly (ADP-ribose) Polymerase-1 (PARP-1) is required for a switch to initiate long-patch BER when the repair product cannot be ligated after incorporation of the first nucleotide by Pol β (Dantzer et al., 2000; Podlutsky et al., 2001; Prasad et al., 2001). In case of long patch repair, Pol β is replaced by Pols 8 or E, which then conduct strand displacement synthesis (Fortini et al., 1998; Stucki et al., 1998)

This long patch repair requires activator like proliferating nuclear antigen PCNA and a 'flap' structure specific endonuclease-1 (FEN1) activity to cut the flap like structure produced by the strand displacement type synthesis by Pol p (Wu et al., 1996; Klungland and Lindahl, 1997). Nealon et al., 1996 suggested that while Pol β is the major base excision repair polymerase in human cells, other polymerases also contribute to a significant extent.

DNA REPAIR IN BRAIN

Neurons are postmitotic and are the longest living cells in the body. The information regarding the ability of the brain cells to carry out specific types of DNA repair reactions is scanty although several DNA repair enzymes have been identified in brain (Waser et al., 1979; Kuenzle, 1985; Walker and Bachelord, 1988; Mazzarello et al.,1992; Rao, 1993; Weng and Sirover, 1993).

Alexander (1967) first noticed that DNA-repair system is at a low key once cells are differentiated in to postmitotic state. Gensler (1981a, 1981b) found that unscheduled DNA synthesis (measure of DNA repair capacity) in response to UV irradiation is markedly lower in mature hamster brain than in mature hamster lung or kidney cells. Korr and Shultz, (1989) demonstrated through autoradiography, that the DNA repair was low in various types of cells of adult mouse brain *in vivo.* Even though the level of DNA repair for some types of damages in neurons is low, this repair might be sufficient to cope with DNA damages if they occur at a low rate in these cells. Studies from this laboratory Subba Rao and Rao (1984) showed measurable levels of DNA Polymerase p, which is considered as repair enzyme in adult and aging rat brain. Further studies also showed there is no change in the fidelity of this enzyme between young, adult and old ages (Subba Rao et al., 1985). A putative 'House keeping' DNA repair enzyme, a non-specific alkaline DNase of rat brain

exhibited high activity during adult and old ages (Subba Rao and Rao, 1982; Subba Rao, 1990). Hanawalt et al., (1992) showed that not only significant DNA repair process occurs in a model neuronal cell system but also the tenet of genomic heterogeneity of DNA repair is applicable to the postmitotic system such as brain.

Brain has different cell types and relatively few studies have examined the expression of DNA repair proteins in different brain cell types. APE/Refl mRNA levels were shown to be particularly high in certain hypothalamic nuclei, as well as hippocampus and cerebellum (Wilson et al., 1996). APE/Refl is a multifunctional enzyme that has both an AP endonuclease activity that is essential for glycolase initiated BER, and also acts as a redox-sensing factor for transcription of fos and jun (Flaherty et al., 2001). Duguid et al., (1995) found that APE/Refl was expressed heterogeneously throughout the brain with high levels in hippocampal neurons. Verjat et al., (2000) using *in situ* hybridization reported heterogeneous expression of 8-oxo- G glycolyase 1(Ogg1) mRNA in different regions of the brain. The highest levels were observed in the hippocampus, cerebral cortex, cerebellum and several hypothalamic and brain stem cell groups.

Le Doux et al., (1996) using primary cultures of homogenous populations of each of the three glial cell types in the brain studied DNA repair and they found that repair of O^6–methylguanine by methyl guanine methytranferase was better in astrocytes than either in oligodendrocytes or microglial cells.

Studies have been conducted to examine DNA repair by mitochondria (LeDoux and Wilson, 2001; Bohr, 2001) The studies indicated that astrocytes have higher DNA repair capacity compared to other glial cell types. Chen et al., (2000) detected base excision repair of oxidative damage in mitochondrial extracts from adult rat brain, as well as from cultured cortical neurons and astrocytes. Mandavalli et al., (2000) found that endogenous

mitochondrial DNA damage in the caudate putamen and cerebellum was higher in 1 year old than 22 day old mice, suggesting an age related decrease in mitochondrial DNA repair,

BRAIN and BER

Brain is very well protected externally (including the blood brain barrier) and it can be assumed that most of damages to the DNA of brain would be due to endogenous factors and from such exogenous factors which can cross the blood brain barrier. There is ample amount of evidence, that base excision repair pathway would be the main guardian to ensure genomic stability in the highly metabolic organ brain. For example Pol p is the enzyme that takes part in the BER when compared to other polymerases found in the nuclei of the mammalian cells particularly in filling the single nucleotide gap (Wood RD, 1997; Wilson III DM and Thompson, 1997; Fortini et al., 1998). Waser et al., (1979) found that Pol β constitutes 90% DNA Polymerase activity in adult rat brain and it was concluded that Pol β is the repair enzyme. It thus can be expected that genomic maintenance in brain cells be largely taken c .e by Pol β dependent BER pathway. As outlined above, many proteins known to be participating in BER pathway are found in brain also (Rao, 2002). The status of BER in health and disease assumed great importance with the accumulating knowledge of several neurodegenerative diseases that appear in old age and their molecular link to the genomic stability (Martin, 1999; Sniden, 2001).

DNA POLYMERASE β

Replication of DNA is carried by enzymes called DNA Polymerases (Brautigam and Steitz, 1998). To day, the number of DNA Polymerases has increased to atleast 19 since the initial discovery of DNA Pol **a** (DNA-Polymerase a) in Eukaryotic cells in 1957. In the early 1970 Pols p and *y* (DNA Polymerase β and DNA-Polymerase y) were discovered,

leading to the simple concept that Pol a was the enzyme responsible for nuclear DNA replication, Pol β for DNA repair, Pol y for mitochondrial DNA replication. Later in 1980's Pol 8 (DNA-Polymerase δ) and Pol e (DNA-Polymerase e) were discovered and intensive work on them suggested that a particular Pol might have more than one functional task and that a particular DNA synthetic event may require more than one Pol (Stucki et al., 2001). In most recent times several other DNA-Polymerases have been discovered (Polymerases η, K, *X*, μ, p etc.,) and these polymerases show the interesting ability of copying the DNA strand with lesions with varied specificity. It is likely that the precise function of these newly discovered polymerases will be known in the next few years (Hubscher et al., 2002).

Pol β (E.C.2.7.7.7) is the smallest eukaryotic Polymerase and it was proposed as a DNA repair enzyme 20 years ago (Hubscher et al., 1979). It is found in vertebrates and lacks intrinsic accessory activities such as 3'-5' exonuclease, endonuclease, dNMP turnover, RNaseH, or the reverse of the DNA synthesis reaction, Pyrophosphorolysis (Baril et al., 1971; Chang and Bollum, 1972; Chang, 1973; Matsukage et al., 1974; Wilson, 1990). The Pol β is expressed independent of the cell cycle stage (Zmudzdka et al., 1988), but the regulation of the enzyme is in a tissue specific fashion (Wilson, 1990). Pol β is composed of a single 39kda polypeptide containing 335 amino acid residues and the secondary structure predictions suggest ordinary globular structure with α-helix content (Zmudzka et al., 1986; Sengupta et al., 1986). Both human and rat enzymes were cloned 15 years ago and extensively studied over the years by Wilson and his group (Zmudzka et al., 1986; Sengupta et al., 1986). The recombinant human enzyme purified from E.coli, does not have exonuclease or endonuclease activity like natural enzymes and recombinant enzyme is similar to the natural enzyme (Abotts et al., 1988). The recombinant enzyme has the same

template-primer specificity as the natural enzyme and has a reactive epitope for anti-p-Polymerase IgG.

Pol β is folded into distinct domains each associated with a specific functional activity. An 8kda amino terminus domain is connected to the 31kda domain by a protease sensitive hinge region (Prasad et al., 1998). These two isolated protein domains have dedicated biochemical activities (Kumar et al., 1990a; Kumar et al., 1990b; Casas Finet et al., 1991; Casas Finet et al., 1992; Prasad et al., 1993; Prasad et al., 1994; Peirson et al., 1996). The 31 kda domain catalyzes nucleotidyl transferase reaction where as the 8kda domain has a lyase activity (dRpase) that removes the 5'deoxyribose phosphate generated after incision by an AP endonuclease during BER (Matsumoto and Kim, 1995) and also single strand binding activity (Prasad et al., 1994).

During the past several years of research evidence has accumulated which confirms a role for DNA Polymerase β in the mammalian AP site BER pathway. Lack of DNA Pol β in DNA Pol β eficient cells or in the presence of neutralizing antibody, a reduction in DNA repair activity is seen which strongly suggests a role for DNA Pol β in BER pathway *in vivo* (Dianov et al., 1999). It was found that Pol β fills up a gap of upto 6 nucleotides in one of the strands of a double stranded DNA very efficiently and in a processive manner if the down stream primer has a 5'-phosphate. On the other hand, if the down stream primer has a 5'-OH or there is no downstream primer at all (no gap at all therefore simply extending the primer using the other strand as template) then the addition of nucleotides to the primer is slow and distributive (Prasad et al., 1994; Singhal and Wilson, 1993). Thus the most preferred substrate for Pol p seems to be a double stranded DNA with a gap of less than 6 nucleotides (the most preferred being single nucleotide gap) with a 5'-phosphate margin at

the down stream primer. It must however be mentioned that Pol β may be slow and distributive, but not inactive, towards simple template primers without any gap in the primer strand (Wang and Kom, 1982). By biochemical and physical experiments the binding site for the 8kda domain is shown to be six nucleotides (Casas Finet et al.,1992) and the intact enzyme covers about 12 nucleotides.

Polymerase p associates with other enzymes of the BER pathway such as DNA ligase I, AP endonucelase, and XRCC1-DNA ligase I. It has been demonstrated (Prasad et al., 1996; Dimitriadis et al., 1998) recently that Pol β and DNA ligase I interact and form a tight complex in solution. Other roles for Pol β have been envisaged (Wilson, 1998). *In vitro* DNA repair studies have shown that Pol p has a role in repair of monofunctional DNA adducts by Hela nuclear extracts (Dianov, et al., 1992) and of UV damaged DNA (Jenkins et al., 1992) and abasic lesions in DNA (Matsumoto and Bohenhagen, 1989) by *Xenopus laevis* oocyte extract. It has also been implicated in meiotic events associated with synapsis and recombination. It dynamically localizes to the synaptonemal complexes formed by chromosomal pairs in meiosis (Plug et al., 1997). The 67kda S.cerevisiae homolog of mammalian Pol p encoded by nonessential Pol 4 gene has been implicated in double strand break repair. It probably utilizes a non homologous end-joining mechanism. Sugo et al., (2000) have shown in mice by targeted disruption of the Pol β gene retarded growth and the mice died of respiratory failure immediately after the birth The increased apoptotic cell death observed in the developing central and pheriperal nervous system suggest that Pol p plays an essential role in neurogenesis.

DNA REPAIR AND HUMAN NEUROLOGICAL DISORDERS OF AGING

DNA repair disorders refer to a group of conditions that are characterized by a failure of distinct cellular DNA repair mechanisms to function properly. The consequences of these failures are far reaching and extend to abnormalities related to normal growth and development, aging (normal and premature), programmed cell death, and cancer inherited conditions. Some of these inherited disorders closely associated with defective DNA-repair are mentioned briefly below.

XERODERMA PIGMENTOSUM (XP)

Xeroderma Pigmentosum is a DNA repair disorder related to the NER repair pathway. It is an autosomal recessive disorder characterized by cutaneous photosensitivity, pigmentary changes, and a propensity for the early development of malignancies in sun exposed mucocutaneous areas, including the eye (Hebra and Kaposi, 1984; Jung, 1986; Kraemer et al., 1987; Brougthon et al., 2002). Photosensitivity and the high cancer incidence observed in Xeroderma Pigmentosum patients are due to the defect in the NER pathway and the resulting genomic instability (Cleaver, 1968; Epstein et al., 1970; Day, 1975; Kraemer et al., 1994; Kraemer et al., 1994; Eveno et al., 1995; Brash, 1997; Kraemer et al, 1997). Most cases are symptomatic in childhood, except for an adult variant form. These symptoms include sun sensitivity, photophobia, and, in about 20% of the patients, neurological abnormalities (De Sanctis and Cacchione,1932; Kraemer et al., 1987; Vermeulen et al.,1994). Mutations in eight different genes have been reported in patients with Xeroderma Pigmentosum. These include genes involved in complementation groups XPA -XPG in the NER repair pathway.

The Xeroderma Pigmentosum variants are deficient in the Polymerase η that allows DNA replication through DNA lesions (Wood RD, 1991; Stefanini et al., 1993; Coin et al., 1999; Cleaver, 2000; Broughton et al., 2002).

COCKAYNE SYNDROME (CS)

Cockayne syndrome is a DNA repair disorder related to the transcription- coupled repair (TCR) repair pathway. It is a progressive neurological disorder characterized in infancy by growth failure, deficient neurological development, progressive retinal degeneration, and sensitivity to sunlight (Cockayne, 1936; Cockayne, 1946; Otsuka and Robbins, 1985). One of the hallmarks of Cockayne syndrome is pigmentary degeneration of the retina, first described by Cockayne in 1936. It also occurs in several types, depending upon the gene that is mutated (Patton et al., 1989; Proops et al., 1981). Type I, "the classical type," has an onset in the post natal period, whereas type II, "the severe type," occurs before birth and usually results in death by the age 6 or 7 years (Nance and Berry, 1992). The Unscheduled DNA synthesis (UDS) is normal in these patients, and there is lack of replicative DNA synthesis after the UV damage in CS cells similar to XP Cells (Lehman, 1987).

ATAXIA **TELANGIECTASIA** (AT)

It is also referred to as Louis-Bar syndrome. AT individuals have defective DNA repair to repair damage caused by ionizing radiation and bleomycin. It is an autosomal recessive genetic disorder that affects many systems of the body, particularly nervous system, immune system and skin. AT cells are abnormally sensitive to killing by ionizing radiation (IR). Patients with AT develop progressive ataxia resulting from atrophy of the cerebellum. The rapid degeneration results in many patients dependent upon wheel chairs before their teenage (Stankovic et al., 1998). Symptoms are seen in early childhood with

progressive cerebellar ataxia and later develop conjunctival telangiectases, other progressive neurologic degeneration, sinopulmonary infection, and malignancies. Dilation of blood vessels, in eye and skin (telangiectases) typically develop between 3 and 5 years of age.

HUTCHINSON-GUILFORD PROGERIA SYNDROME (HGP)

HGP is an extremely rare genetic disease that accelerates the aging process to about seven times the normal rate. Because of this accelerated aging, a child of ten years will have similar respiratory, cardiovascular, and arthritic conditions that a 70-year-old would have. Currently, there is no cure for this disease, and because of its rare nature, no definitive cause can be pinpointed. Some physical features of Progeria children include dwarfism, wrinkled/aged-looking skin, baldness, and a pinched nose. Mental growth is equivalent to other children of the same age. Most children with Progeria live no longer than their early teenage years. Cultured HGP fibroblasts have been reported to have decreased ability to repair single strand breaks following gamma irradiation (Epstein et al., 1973; Epstein et al., 1974).

WERNER SYNDROME (WS)

Mutations in the RECQL2 gene, encoding for a DNA helicase, are responsible for Werner syndrome. (Gray et al., 1997; Nehlin et al., 2000; Mohaghegh and Hickson, 2001; Shen J and Loeb, 2001). Werner syndrome is characterized by caricatural premature aging associated with graying of the hair often before the age of 20 years. Malignancy occurs in 10% of the cases. The features of Werner syndrome are scleroderma-like skin changes, especially in the extremities, cataract, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, and a wizened and prematurely aged faces. Fujiwara et al., (1977) showed that the elongation rate of DNA chains during replication was significantly slower in WS skin fibroblast cells than in normal cells. These cells exhibited normal repair of X-ray

induced and single strand breaks and UV induced repair synthesis. The finite replicative life span of human cells *in vitro*, the Hayflick phenomenon (Hayflick, 1965) is due to the stochastic loss of replicative ability in a continuously increasing fraction of newborn cells at every generation. Normal human fibrobiasts achieve approximately 60 population doublings in culture, while Werner syndrome cells usually achieve only about 20 population doublings (Faragher et al., 1993).

BLOOM'S SYNDROME (BS)

Bloom syndrome is due to mutations in the RECQL gene, a DNA helicase involved in DNA replication and repair (Ellis et al., 1995; Karow et al., 1997; Kitao et al., 1999). Bloom syndrome is characterized by growth deficiency, variable degrees of immunodeficiency, and predisposition to cancers of many sites and types (German, 1995). Patients with this disease show a range of symptoms which include a small body size, sun-sensitive facial reddening, sub- or infertility, immunodeficiency and a predisposition to the full range of human cancers. Cells from patients with Bloom's syndrome are genomically unstable and show elevated levels of both homologus recombination and sister chromatid exchange.

FANCONI'S ANEMIA (FA)

Fanconi's anemia is an inherited autosomal recessive disorder. It is classically diagnosed between 2 and 15 years of age. The disease is caused by a genetic defect that prevents cells from fixing damaged DNA or removing toxic, oxygen-free radicals that damage cells. It is characterized by refractory anemia progressing to pancytopenia, congenital and developmental abnormalities, and an increased incidence of malignancy. Fanconi cells are deficient in repair of dihydrooxydihydro thymine residues, hypersensitive

to cis-platinum (Fujiwara et al., 1987), DNA cross linking agents like mitomycin C (Fujiwara et al., 1977).

ALZHEIMER'S **DISEASE** (AD)

AD is a degenerative disorder of the central nervous system in humans. It is characterized by progressive neuronal degeneration, which is regarded as a feature of accelerated aging. In AD, neurons in cerebral cortex, basal forebrain, and locus ceruleus are progressively lost. There are evidences to suggesting reduced repair of some types of DNA damage (Ionizing radiation, DNA damaging alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine etc.,), a decline in gene expression in the brain, and cellular degeneration in a specific region of the brain. Sensitivities of DNA to ionizing radiation was shown from a series of unrelated AD individuals where majority of them showed significant greater sensitivity than cells from age matched control donors (Kidson and Chen, 1986). Scudiero et al., (1986) reported that AD cells showed small but statistically significant hypersensitivity to the DNA damaging alkylating agent MNNG (N-methyl-N'-nitro-N-nitrosoguanidine). At present AD cells were reported to be defective in repair of X-ray damages by some investigators (Kidson and Chen, 1986; Robbins et al., 1983a; Robbins et al., 1985). But there are reports contradicting the above studies (Smith et al., 1987; Smith and Itzhaki, 1989)

PARKINSON'S DISEASE (PD)

PD is characterized by progressive degeneration of the central nervous system in elderly. Both AD and PD are sporadic disorders. In PD, Neurons in the substantia nigra, basal forebrain, and locus ceruleus are progressively lost. Robbins et al., (1983a) found that cell lines from six patients with PD were significantly more sensitive to X-rays than were normal cell lines. Sensitivity to UV irradiation was normal in these patients. These results

suggest that such a DNA repair defect could cause rapid abnormal accumulation of spontaneously occurring DNA damage in PD and AD neurons *in vivo*, which results in premature death.

More than 150 human genetic disease syndromes have been characterized as having some potential relationship to the normal biology of aging. Approximately, 40% of infant mortality results from genetically determined conditions (Childs, 1975).The great abundance of human genetic variations raises the possibility that certain mutations will effect genes concerned with longevity. Although we know of no single mutation that lengthens maximum life span, it is apparent that a number of mutations shorten life. Whether or not any of these life shortening mutation reflect alterations in some of the genes that might relate to longevity is unclear.

In some syndromes evidence of both elevated DNA damage and premature aging is observed. These include Ataxia talengeitasia, Cockaynes syndrome, Werner's Syndrome. Neurodegeneration is seen in Ataxia talengeictasia, Cockaynes syndrome, Xeroderma pigmentosum, Huntingtons disease, Parkinsons disease, Alzheimers disease.

SCOPE OF THE PRESENT STUDY

The present study constitutes the continued efforts from this laboratory to assess the validity of the hypothesis that accumulation of DNA-damage and decreased DNA-repair capacity is at least one of the major naturally chosen genetic switches for initiating the phenomenon of aging and its associated disabilities. The emphasis of this work is of course on the brain cells.

1. To day several DNA Polymerases performing various functions contributing to the overall DNA-replication and maintenance of its structural integrity are known. Some of these polymerases are discovered in only in recent past and it is likely that this number

might increase in future. However in a post mitotic cell like neuron, there are reports to indicate that Pol p is the only polymerase almost exclusively present. Studies from our laboratory have also revealed that Pol β is the most predominant DNA Polymerase in the rat whole brain. We have now undertaken to examine the DNA Polymerase activities in extracts of neuronal and astroglial cells fractions from the rat cerebral cortex at three different ages. The results showed that the while Pol β is the most predominant DNA Polymerase in these brain cells at all the post-natal ages, some activity attributable to Pols α,δ,ε is also present. These results are presented in the chapter 3.

2. The relationship between DNA repair and phenomenon of brain aging has been subject of study in this laboratory for the past several years and (BER) accounts for the main DNA-repair mechanism in brain cells and Pol β being a main player of that pathway. Earlier results from this lab pointed out that with the advancement of age, not only the levels of Pol p come down but also there is accumulation of the catalytically incompetent Pol β molecules. In order to show that the Pol β dependent DNA-repair is the one that is compromised in brain cells during aging, we have taken up a more *in vivo* relevant functional assay for Pol β activity. We have therefore, chosen a simple and 'easy to work with' model for measuring the Pol β activity in aging neuronal extracts. The results of primer extension activity with age indicate that the activity of Pol β in brain cells is compromised with age and that this deficit can be corrected *in vitro* by addition of pure recombinant rat liver Pol β under appropriate condition. The above studies also indicated the presence of 3'-5'exonuclease activity (proof reading activity) in neuronal extracts which was found to be facilitating the extension activity Pol p particularly with respect to primers with a mismatched base at the 3'-end. We have

carried out the exonuclease activity assays in order to know the status of 3'-5' exonuclease activity in brain cells with advancing age of the animal since this activity could become a constraint for proper functioning of Pol p. These results are presented in chapter 4.

3. It is reported that Pol β acts most efficiently in filling up a gap of single nucleotide and can also fill up upto six nucleotides in a relatively processive manner. Therefore, investigations were continued to examine the gap filling repair activity in aging neuronal extracts with an appropriate model oligo substrate. The results indicate that the gap filling activity is very low in adult and old neuronal extracts. Supplementing these neuronal extracts with recombinant Pol p restored the gap repair activity predominantly by slow distributive strand displacement manner. These results are presented in chapter 5.
4. In chapter 6 all the results presented in earlier chapters have been discussed in the light of the exi ing information.

Objectives of the study

1. To study base excision repair pathway with a synthetic oligo model system in Young, Adult, and Old neurons.

2. To Examine the ways and means to bring back the lost activity (if any) in aging neurons to normal level.