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Can Medical Research Help Modulate The Rate of Aging?

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Abstract

Research to understand the mechanisms behind attaining old age and senescence has received great impetus during the past 2 decades. While the inevitability of the process and the end is obvious, many interesting features of this evolutionarily opted phenomenon are being surfaced. Aging and life span of species seem to be influenced both by genetic component as well as environmental forces. It is also apparent that life span of species has a relation to the time taken to reach reproductive maturity. Work with model organisms and some human syndromes characterized by accelerated aging has indicated that there are about 250-300 genes that are closely linked to the process of aging. Any subtle changes in the expression of these genes seem to significantly alter the rate of aging and life span. Most of these genes are found to code for pathways related to energy and stress handling metabolism and maintenance and repair mechanisms. Among the maintenance and repair pathways, DNA repair mechanisms, in particular those pathways like base excision repair pathway vested with the function of repairing 'in house' damage to DNA are emerging as important modulators for the aging phenomenon and associated debilities like neurodegenerative disorders. These advances in aging research are

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also becoming initiators for a hope to achieve a considerably lengthy health span in human populations.

Key Words: Aging, DNA repair mechanisms, genetic component, environmental forces

Introduction

History has never witnessed so much information and knowledge on human body and the molecular mechanisms that control its miraculous functioning, as it is now. The two fundamental and awe inspiring natural phenomena, the birth and death had so much influence on human civilization and attracted the capabilities of the best minds to understand these phenomena. Medical science has gained some significant ground in the case of former. Today the detailed knowledge about how a baby is conceived, the kinetics of its growth in mother's womb, the delivery of the baby and the requirements for the growth of the delivered infant, is available. As a result of this entire advancement one is able to control the whole process and decide when to have a baby. Even the sex of the baby to be born can be ascertained through appropriate scanning procedures.

However, it has not become possible for scientists to display the same confidence as far as the second phenomenon mentioned above, the death, is concerned. Even then, the progress in the medical sciences in the areas of nutrition and healthcare has achieved improved life span of people in general although there seems to considerable difference between advanced countries as compared to the developing nations. In India too the average life span has increased from around 42 at the time of independence to 63 as of today. It is generally believed that this is due to significant decrease in infant mortality coupled with improved health care which has become affordable for more number of people. People are living longer and with the percentage of people above 60 in India reaching the double digit, this section of population started drawing the attention of the government, the planners and more importantly the researchers. Little is known about why we become old and then die. It is a challenge that greets this millennium. It is a challenge whether the rapid advances in science and technology would enable maintenance of good health and the postponement or reversal of that type of old age marked by debilitating diseases, frailty and senility. Would it ever be possible to

preserve youthfulness for the later years in life?

It is the purpose of this article to present an update on the status of Science research seeking to explain the molecular mechanisms behind the aging process. Further, to point out the leads that this research has provided to offer hopes to modulate, if not gain complete control, the process of aging. Finally, the very modest contribution made from the laboratory of this author towards a slightly better understanding of the DNA Repair mechanisms in brain and its tight relationship to aging and age associated neurodegenerative disorders will be mentioned.

Molecular biology of Aging

Up until 50 years ago, research emphasis on the biology of aging was scanty. However with the advent of molecular biology, research in medical sciences and healthcare improvement coupled with good nutrition resulted in extended longevity of populations particularly in developed countries. With this, certain debilitating disorders like Alzheimer's and Parkinson have started making their appearance more frequently in these aging populations. Living longer is fine but it became clear that it brings in some associated problems to the society and to the government committed to the welfare measures like providing medicare to senior citizens. Intensive research began to understand the mechanism behind getting old and associated debilities; both out of curiosity and necessity.

There were theories galore to explain why living organisms age and die. As the research went along, several questions emerged that need to be addressed. For example, primitive unicellular organisms that multiply by binary fission have no death. Each cell keeps on dividing as long as nutrition is available and practically no part of the parental cell seems to have been lost during vegetative division. However, death of the parental cell/organism has come into operation when the evolution opted for sexual reproduction (1). It appears that while sexual reproduction offers the hybrid vigor to the offspring, the price for this is parental death at different points of time after the sexual act depending upon the species and their potential longevity. Another striking observation of the researchers is the average life span of different species which must be a product of genetic as well as environmental contributions. The average life span of some species is given in Table 1 to emphasize this point. What is interesting is the apparent positive correlation between the times taken to reach adult hood (reproductive maturity) in any given species, to the length of the life span of that species. This gave an important clue about the

network relationship between the attainment of reproductive maturity, the life span and the aging process which supposedly begins from the onset of reproductive maturity.

Table 1 : Longevity and Time taken for Reproductive Maturity

Species	Longevity (Years)	Age of Puberty (Years)
Humans	100	12-14
Elephant	70	12-14
Chimpanzee	40	10
Dog	30	1
Rhesus Monkey	25	3
Cat	25	1.5
Rat	3	0.25
Mice	3	0.20

In spite of the fact that some evolutionary biologists along with their experimental scientist friends vehemently argue that there are no special genes coding for aging process and that human longevity is made up by genes only to an extent of 25 % (2, 3) there is overwhelming evidence accumulating over the years that genetic component exerts a major influence in determining the longevity potential. For example microsomal transfer protein gene located on chromosome 4 has been identified as a longevity contributor in a group of centenarians(4). Further the genetic component in centenarians is increasingly becoming apparent from studies on human populations(5,6). To cap it all, as of today, there are about 280 genes that have been identified in various organisms that influence the life span one way or the other(7). The most extensively studied organisms include *C.elegans*, Drosophila, yeast, and mice. For obvious reasons information from human studies is scanty or indirect. However, knowledge from the homologous genes, cell culture studies and last but not the least from various progeroid syndromes characterized by accelerated aging in humans, *viz.*, Werner's syndrome(WS), Hutchinson Gilford Progeroid syndrome(HGPS), Down syndrome (DS), Bloom's syndrome (BS), Cockayne's syndrome (CS) and Ataxia telangiectasia (AT) is helping to understand the genetics of aging process.

In Table 2, the numbers of genes in different organisms that are known to exert influence on rate of aging process and therefore on the life span of those species, are listed. The numbers indicated are approximate as the information is constantly undergoing revision with newer findings. The present data are obtained largely from the data base available at University of Washington, Seattle web site (7). Some genes, when mutated, are

known to extend the life span while the other shorten the life span. These numbers are also indicated in the table to highlight the evolutionary trend where in the number of genes that could extend the life span decrease in more advanced species. For example in humans, as on today, there appears to be no genetic disorder that would result in extended life span!

Table 2 : The number of genes,when mutated, known to either extend or shorten the life span in different species.

Organism	Total Number of genes Known to modulate life span	No of genes that shorten life span	No. of genes that extend life span
C.elegans	116	12	104
S.cerevisiae	94	48	46
Drosophila	35	5	30
Mice	24	13	11
Humans	8	8	NIL

Information gathered largely from University of Washington data base (7)

The extensive genetic data mentioned in Table 2 have been carefully analyzed by many scientists to examine whether or not all those genes that have been found to have some relation to life span would fit into any discrete metabolic pathways. An excellent review has appeared most recently on this subject(8) and it turns out that most of the life span modulating genes would fall into 3 or 4 metabolic/signaling pathways. In general the accumulated information, indicated in Table 2 already reveals that while moving up the evolutionary ladder, the genomic complexity increases and the number of genes with a clear impact on life span decreases. Nevertheless, there seem to be a general trend emerging in that life span determining genes are generally involved in certain metabolic and signaling pathways while the interactive network may be more complex in higher species like humans. The following pathways seem to be very intimately involved with the process of aging and therefore the life span.

1. Insulin/Insulin like growth factor-1(IGF-1) signaling :

Originally, the genetic regulation of life span has become evident from the studies on the nematode, *C.elegans*. Mutations in genes like daf-2 and age - 1 resulted in extended life span in these worms (9). There was homology between these genes and mammalian genes encoding Insulin receptor and IGF-1 receptor and phosphatidy-linositol-3-OH kinase (10, 11). It is now certain that even in higher animals like mice and humans the insulin signaling pathway coupled with growth hormone releasing hormone and growth hormone are closely linked to the life span. Inhibition of this pathway results in increased life span (12, 13). It is interesting that mutations in IGF-1 receptor with low plasma IGF-1 levels were found in Italian centenarians (14). Thus glucose utilization pathways seem to have profound effect on the rate of aging and life span.

2. Stress related pathways:

One of the major stresses that all the oxygen dependent organisms have to live with is the oxidative stress. The very energy metabolism in mito-chondria would produce reactive oxygen species (ROS) and therefore oxidative stress on the cell but usually this is handled by two important enzymes, catalase and superoxide dismutase (SOD). In mammals three SOD genes have been identified for handling the oxygen derived stress. Catalase and SOD2 (Mn-SOD) variant seem to be more important and related to aging and life span (15). There is also a claim that over expression of SOD2 in mice leads to increased life span. Reactive oxygen species and such other chemicals can, if not removed, cause damage to macromolecules including DNA and this can have adverse effects. Apart from the oxidative stress, other forms of stress are common in all the higher organisms. Cells suffer from high temperature stress and also endoplasmic reticulum stress due to improper folding of proteins. Heat shock proteins and such other factors are considered to take care of these stressful situations. Further cardio-vascular stress particularly in individuals with advancing age plays a major role in the aging process and longevity determination. Apo lipoprotein E variants are known to be important players in human ailments involving cardiovascular stress and neurocognitive deterioration (16). The ability to handle stress is tightly linked to aging process (17).

3. Macromolecular repair and maintenance mechanisms:

During evolution, organisms have developed various strategies to repair any damage that may have occurred to

any of the cellular components including the macromolecules like DNA, RNA, proteins and membrane structures. The most important of all these strategies is perhaps, the ability to repair damaged DNA, so essential to maintain the structural integrity of DNA so that faithful transfer of genetic information from generation to generation is assured. A number of DNA repair pathways have been identified in both prokaryotes and higher organisms. In mammalian cells four major DNA repair pathways are identified: (1) A simple reversal of the damage, (2) Nucleotide excision repair (NER) including mismatch repair and transcription coupled repair, (3) Base excision repair (BER) and (4) Recombinational repair (RR) including non homologous end joining repair (NHEJ). It is beyond the scope of this article to discuss these pathways here. However, informative and critical reviews have appeared in recent times and these may be referred to for more information (18-22). There are a couple of reviews specially dealing with DNA repair pathways in brain (23, 24). Suffice it to say here that all these pathways and in particular the NER and BER seem to have a tight relation to the phenomena of aging and neurodegenerative diseases (25-27). Furthermore, BER is the pathway that appears to be eminently equipped to handle the simple damage that occurs to the bases in DNA as a result of very cellular metabolism itself that is known to generate reactive oxygen species. Spontaneous deamination and loss of DNA bases could also occur eventually resulting in mismatched base pairs and baseless sites. In view of this BER is being considered to have a major role in maintaining the structural integrity of genomic DNA and therefore to the processes like aging and neurological disorders (24, 28-32).

In view of the special interest of this author in brain aging and associated neurological disorders and their relation to BER pathway, this pathway is briefly outlined in Fig 1. Essentially there are four steps in this pathway and two sub pathways could be visualized, one concerned with 'short patch or single nucleotide replacing pathway' and the other 'long patch pathway' involving filling up of a patch of up to 13 nucleotides. Some details about the steps are also presented in the legend for Fig 1. In recent years, BER is drawing the main attention as one of the most crucial DNA repair pathways for the following reasons: (1) This is a pathway which is conserved as well as evolved through the evolution to take care of the DNA damage concerned with loss or modification of the bases that are likely to happen in the normal course of cell's life and

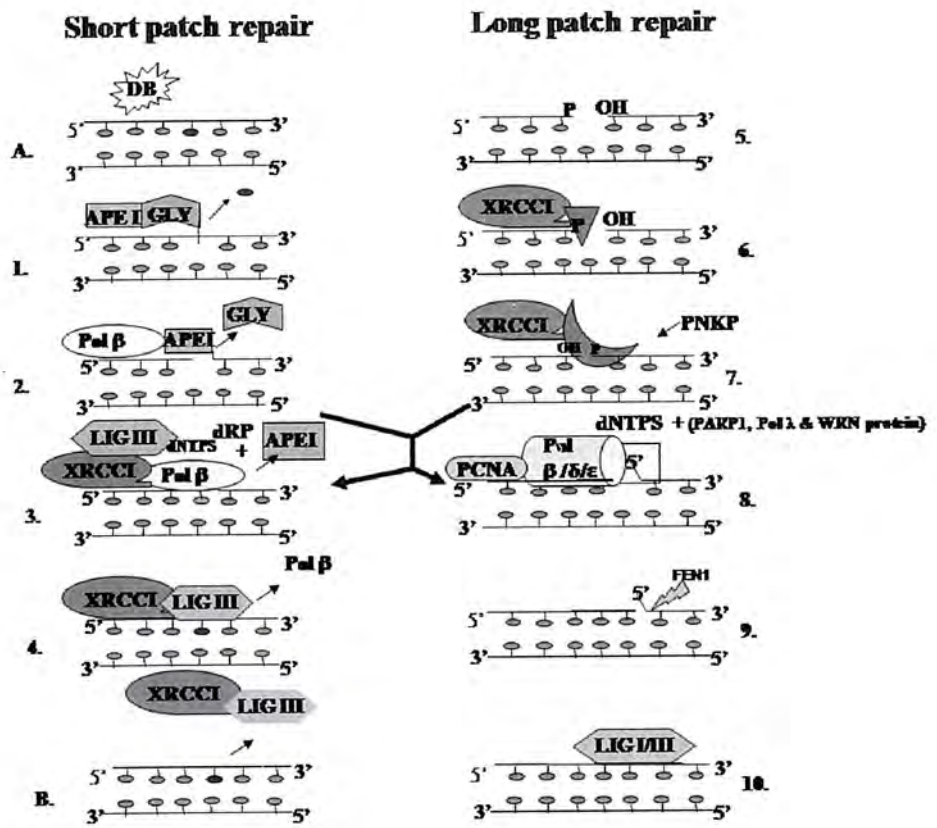

Fig 1: Base Excision Repair (BER) Pathway. On the left and right side are the 'Short patch' or single nucleotide pathway and the 'long patch' pathways respectively. Crossing over of the pathways can occur at points 2 and 7.

In step one of short patch pathway (left panel) the altered base (A) is recognized and cleaved from the deoxyribose phosphate moiety by an appropriate DNA-glycosylase. At the same time, the AP endonuclease (APE1) reaches the site. The second step consists of breaking the chain at 5'-side of the abasic site. The major endonuclease specific for abasic site in DNA in humans is APE1. In step 3, the pol β fills up the one nucleotide gap and also releases the 5'-deoxyribose phosphate (dRp). At the same time DNA-ligase III - XRCC1 complex arrives at the site. Step 4 consists of DNA-ligase III sealing the nick and pol β dissociating from the site. Subsequently the XRCC1 and ligase III come off from the site leaving behind repaired DNA (left panel, B).

The predominant route for BER is the 'short patch or single nucleotide pathway' discussed until now and shown on the left panel. In cases where the terminal sugar phosphate after the AP endonuclease incision (Step 2) develops a complex structure that cannot be acted

upon by the dRpase activity of pol β (for example, oxidized abasic site) the repair synthesis would nevertheless continue but in a strand displacement manner (right panel) This long patch synthesis is catalyzed either by pol β itself or a bigger polymerase like pol δ/ε with associated proof reading activity. Also, this pathway is stimulated by PCNA and requires a 'flap' structure specific endonuclease-1 (FEN1) activity to cut the flap like structure produced by the strand displacement type of synthesis by pol β. PCNA seems to stimulate the FEN1 activity and the repair patch size is about 7 nucleotides. The ligation can be achieved; it appears, either by DNA-ligase I or III. At this time, it is a matter of speculation as to what determines the type of DNA polymerase to be recruited for long patch BER pathway. The involvement of four other proteins in BER is reported in literature. These are PARP-1, PNKP, DNA-polymerase lambda (polλ) and Werner syndrome protein.

Abbreviations: DB-damaged base; APE1-human apurinic/apyrimidinic endonuclease 1; GLY-DNA-Glycosylase; Pol β/δ/ε- DNA-Polymerase β/δ/ε respectively; dRP-deoxyribose-5'-phosphate; XRCC1-X-ray repair cross-complementing, gene1; LIGI/III- DNA-ligase I / III; PARP1-Poly (ADP-ribose) Polymerase1; PNKP- Polynucleotide Kinase 3-prime phosphatases; FEN1- 'Flap' structure specific endonuclease 1; dNTPs- deoxynucleoside triphosphates. There is some recent suggestion that one of the several DNA-polymerases being discovered newly, the DNA-polymerase lambda (λ) has similar properties as that of pol β and may participate in BER. Figure taken from reference 28.

metabolism, (2) The relatively high importance of this pathway in post mitotic organs like brain where high oxygen dependent metabolic activity is seen in spite of lack of cell replication and (3) Being recognized as main pathway for repairing the oxidative damage to DNA bases.

In our laboratory, we have measured the activity of DNA polymerase β, a crucial enzyme in BER pathway, in rat neurons at different ages of the animal. The findings are summarized in Table 3. As can be seen, the activity of this important enzyme markedly decreases by adult hood itself with further decrease at the old age. In the subsequent studies we have examined the most crucial step in BER pathway, the gap filling activity after the damaged base is removed (a combination of steps 2 and 3 in the left panel of Fig 1). For this, synthetic oligo duplexes (32-mers) with one or four nucleotide gap in one of the strands are used as substrates and the gap filling activity in young, adult and old neuronal extracts is measured (Fig 2). Gap repair involves two steps: the creation and filling of the gap by the addition of the required number of nucleotides followed by the ligation

Table 3 : DNA Polymerase activity in rat neuronal and astroglial cells of different ages with 'Activated Calf Thymus DNA'

SUBSTRATE		AGE	
	YOUNG (5 days postnatal)	ADULT (6 months)	OLD (28 months)
'Activated DNA'			
Neuron	2023±1076	719 ± 541*	568 ± 412*
Astroglia	1471± 550	822 ± 512*	694 ± 652*

Values are averages ± S.D. and expressed as picomoles of the radioactive deoxynucleotide incorporated into the acid insoluble fraction in 1hr/mg protein.

*These values are significantly different ($p<0.001$ for neurons and 0.02 for astroglia) from the corresponding value at "young". Data taken from ref.24 and 33

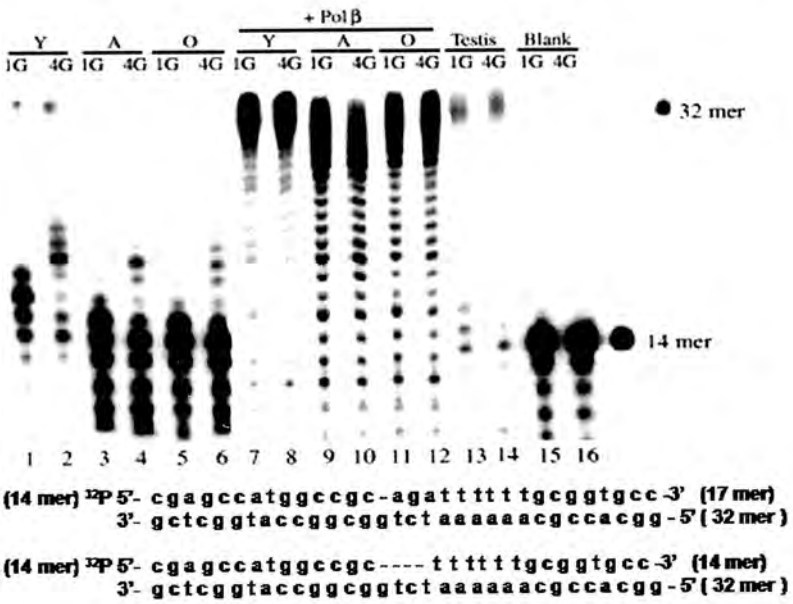

Fig 2: Gap repair activity in 'Young', 'Adult' and 'Old' neuronal extracts supplemented with recombinant pure rat liver pol β .

A typical autoradiogram from three different experiments is shown. Lanes 1-6 neuronal extracts from young brain (Y, 5 days postnatal), adult brain (A, 6 months) old brain (O, 2 years) Lanes 7-12 neuronal extracts supplemented with 1 unit of pol β. Lanes 13 and 14 are

with testis extracts alone as positive control. Lanes 15 and 16 are without any neuronal extracts (Enzyme blanks). The mobility of labeled standard 14-mer and 32-mer are also shown.Lanes 1,3,5,7,9,11,13,15 are with 1-gap substrate(1G) while lanes 2,4,6,8,10,12,14,16 are with 4-gap substrate(4G). The oligoduplexes with 1 and 4 nucleotide gap used in the study are also shown. Figure taken from ref 34.

with the 5'-phosphorylated downstream primer. This repair process, if completed properly should give a radioactive spot on the sequencing gel corresponding to the 32-mer since one of the deoxynucleotide triphosphates used in the reaction mixture is 32P labeled. However no addition of nucleotides to upstream primer (14-mer) was seen in adult and old extracts (Fig 2). In the young, some addition of nucleotides was seen and ligation to downstream primer also occurred although quite feebly. On the other hand, when the extracts were supplemented with pol β, addition of nucleotides occurred all the way to extend the upstream primer to a 32-mer apparently in a distributive strand displacement manner. It was found that excessive amounts of pol β would result in strand displacement type of addition of nucleotides while low amounts of pol β would add just the required number of nucleotides. Even then ligation was achieved only in young extracts and no ligation could be visualized in adult and old (Fig 3). Finally efficient gap filling followed by ligation, that means complete gap repair, was achieved and for this to happen, conditions required are the presence of 5'-PO_4 on the downstream primer, and supplementation of aging neuronal extracts with both pol β and DNA ligase (Fig 4). These studies thus demonstrated that aging neurons are unable to affect BER due to deficiency of pol β and DNA-ligase and fortifying the neuronal extracts from aged animals with these two factors can restore the lost BER activity.

Some of our most recent experiments confirmed the above results with gap filling activity with respect to overall BER activity as well. The overall BER activity is drastically reduced in neurons of aged animals and the activity could be restored back to a significant extent by the supplementation of aging neuronal extracts with pol β and DNA ligase, an observation similar to that with gap filling activity. Further, our very recent experiments also showed that limited (40%) dietary calorie restriction initiated in adult rats and continued upto old age (2 years) resulted in improved pol β activity in neurons (recent unpublished observations). In view of the importance of BER activity in a tissue like brain

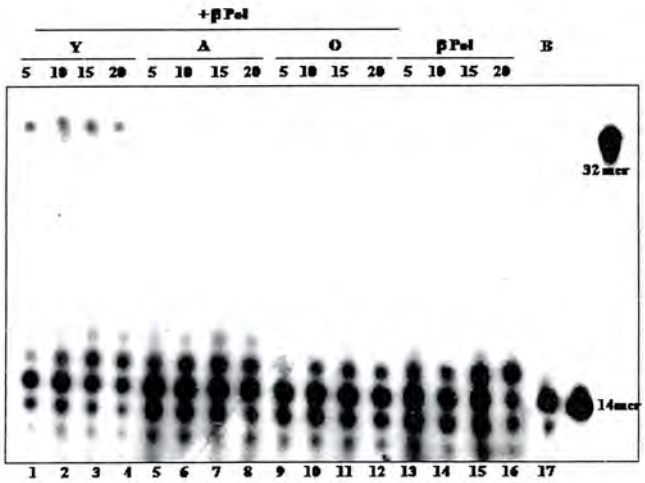

Fig 3: Time course of Gap repair activity with 'Young', 'Adult' and 'old' neuronal extracts supplemented with *low amount* of recombinant pure rat liver pol β (0.2 units)

A typical autoradiogram is shown. Lanes 1-12, neuronal extracts from young brain(Y), adult (A) and old (O) rat brain with 0.2 units of added pol β. The incubation times in minutes are also indicated above each lane. Lanes 13-16 are with pure pol β only. Lanes 17 is without any neuronal extracts (Enzyme blanks). The mobility of labeled standard 14-mer and 32-mer are also shown. Other details and notations are similar to the Figure 2. Only 1 gap duplex was used as substrate. Figure taken from ref 34.

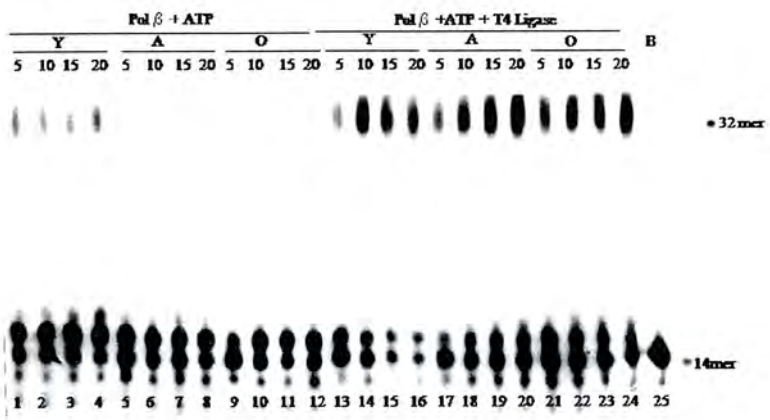

Fig 4 : Restoration of the gap repair activity in adult and old rat neuronal extracts when supplemented with limited amounts of pol β (0.2 units) and 20 units of T4 DNA ligase.

All the experimental details and notations are as in figures 2 and 3.

and the role of DNA repair in maintaining genomic integrity and therefore sustaining the health span of the individuals, these observations are refreshing in the sense that attempts can be made to extend these *in vitro* observations to an *in vivo* situation.

Epilogue

Aging and death are the products of biological evolution influenced by both genetics and environment. At this point of evolution, both these phenomena are inevitable. Yet when science started looking at these processes and the molecular mechanisms behind them, it has become one of the most fascinating journeys of scientific research. Today, even though so much information has accumulated about the process of becoming old, yet it is not clear why there exists so much diversity in life spans in different species. The precise molecular relationship between the reproductive system and the life span is yet to be understood. While many individual genes have been identified in model organisms like *C.elegans*, drosophila, yeast and mice and sometimes a mutation in a single gene is known to extend or decrease the life span of the model organism, the situation in humans seem to be much more complicated and extrapolation of observations from lower organisms to higher organisms must be done with caution. It is indeed interesting that if one looks up the evolutionary ladder, the dramatic influence of single genes on life span seem to be distinctly disappearing(Table 2). It is becoming clear that in highly evolved species like humans one may have to look for system analysis of discrete pathways rather than single genes.

Be that as it may, research on aging has reached a critical stage where it began to identify certain metabolic pathways that are likely to influence the rate of aging and the extent of life span. Two or three pathways are emerging as crucial in this context: (1) The efficiency of glucose utilization for various purposes including energy, (2) Pathways related to handling stress of various kinds including oxidative stress and (3) Maintenance and repair pathways like DNA repair and protein folding. Among these, the glucose utilization and DNA repair pathways have been receiving high attention and perhaps for valid reasons. Glucose metabolism is related to both energy and production of reactive oxygen species. DNA repair is related to maintenance of genomic stability for proper information transfer. If DNA damage accumulates and DNA repair potential diminishes with age then it is to be expected that a chaotic situation

would develop in cellular events leading to cell death and aging among other things. Whether this is nature's design to trigger senescence and death is a matter for pondering. What is more important is the fact that increased understanding of these pathways would lead to capacity to modulate these processes and therefore to achieve, perhaps, a lengthy health-span in human life.

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