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Mechanism of Aging

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Aging is a progressive deterioration of various functions that is accompanied by decreasing fertility and increasing mortality after an organism attains reproductive maturity. Why an organism, after attaining an active adulthood, undergoes deterioration of functions, ages and dies is a challenging problem for Biologists. Several experimental models such as rat, mice, bird, insect, free living nematode and yeast, and cells grown in *in vitro* culture, have been used to study the biochemical, molecular and genetic changes that occur in organisms after they attain adulthood. Several theories and mechanisms have been put forward to explain the molecular basis of aging at the level of genes. For example, random damage of the DNA of somatic cells is believed to accumulate with increasing age and inactivate genes leading to deterioration of functions. Free radicals produced during oxidation of metabolites for energy production also damage DNA and proteins. Mutations of specific genes have been shown to affect specific functions and longevity. Besides some constitutive genes which are active in all organs, some specific genes are involved in the functioning of each organ as it performs specific functions. There is a decline with age in the expression of both types of genes in an organ-specific manner. There are, however, some genes which may be up-regulated and a few others which do not show any change. There are also variations in the time of occurrence and rate of such changes in the organs among individuals of a species. Hence there are variations in their rate of aging and longevity. This shows that aging is not due to a single gene, and it is not programmed. Rather the normal aging process and longevity of a species may be due to destabilization or break down of the homeostatic functioning of the genes that maintain adulthood due to various types of stresses that the organism encounters during its adult life. It may be possible to prevent deterioration of specific functions as it has been shown that caloric restriction not only gives protection against free radicals but also extends longevity. Moreover, decrease in the expression of several genes has been reversed by administration of hormones and other effectors. However, it has to be realized that aging is multifactorial and depends on the expression of several genes in a tissue-specific manner. Identification of these genes, and development of methods by which their expression can be regulated are necessary before attempts can be made to prolong adulthood or defer the onset of aging of an organism.

Key Words: Aging, Mechanism, Somatic mutation, Free radicals, Genetic changes, Gene regulation, Caloric restriction, *in vitro* aging, Telomere

Introduction

Human life span or longevity has been increasing continuously since the beginning of the 20th century, especially after the discovery of antibiotics and advances in medical sciences and health awareness of people. In India, at the time of Independence in 1947, our average life span was about 32 years. Now it is over 60 years. Nearly 7% of the Indian population is now over 60 years and comprise elderly people. The Indian population reached 100 crores (1000 million) on May 11, 2000. So we then had 7 crores (70 million) of elderly. Within one year (by May 11, 2001), the population increased by nearly 15 lakhs (1.5 million) of whom one lakh (0.1 million) are elderly. At this rate we shall have over 12 crores (120 million) of 60+ elderly by the year 2025. In the developed nations the situation is more serious as nearly 12 – 15% of the population is over 60 years. In USA, in 1900, 4% were over 65; in 1998, 13%.

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The problem facing all nations is how to improve the quality of life of the elderly population so that not only they live happily but also their burden on the society and the Government decreases. Unfortunately, after 60 when most people become jobless and unoccupied, several health (medical), social and financial problems arise. Also their physical and mental abilities decline and they become increasingly susceptible to various types of old age diseases: heart and kidney problems, diabetes, cancer, arthritis, asthma, depression, Alzheimer's disease, etc. The importance and seriousness of the problem can easily be realized.

Hence it is essential that we understand why we get old, that is, what is the mechanism of aging? Elucidation of the fundamental cause of aging which occurs in all organisms including humans, is an intellectually challenging problem in Biology. Unravelling the cellular, molecular and genetic basis of this phenomenon may help us to postpone the onset of aging, or slow down the process of aging, if not stop it altogether. This will prolong the active and youthful period of humans from say age 20 - 40 yrs at present to 20–60 yrs or more, and improve the quality of life. We shall add life into years, not merely years to life. We may ensure better health for a longer period as longevity increases. This would postpone or defer the onset of 'old age' diseases which occur from about age 40–50 when the persons are at the peak of their careers. Longer adulthood would not mean increase in population as by increasing literacy and adopting birth control measures it has been possible to gradually reduce both the birth rate and the population structure of a nation.

Aging is described as a progressive deterioration of various functions of organisms after they attain reproductive maturity and adulthood with a simultaneous increase in the probability of mortality with advancing age. Adaptability of the organism to various types of stress declines, increasing the chances of mortality. In humans this decline begins long before the age of 60 and becomes perceptible generally from age 40–50. The important questions that need to be answered are: why do these functions decline after we have attained a vigorous and active adulthood, and why our adaptability to various stresses decline? Is there a trigger, a switch (a gene) which sets in motion the process of deterioration? If so, how and when it is switched on? Or, there are multiple switches (genes) which get switched on at a certain phase of life, and set in motion the process of deterioration of various functions of the organism leading to aging and death? Is this process programmed like the process of development? Answers to these questions would not only help in unravelling the mechanism of aging, but also help in developing methods to postpone the onset of the aging process, thereby prolong the youthful and active period of life.

We all do not age the same way. A man of 60 may look like 65, and another may look like 55. This is not only because of the differences in our genetic make up but also due to the types of nutrition we take, and the types of stresses like psychological stress, temperature, pollution, radiation, etc. to which we are exposed to during our developmental and adulthood periods that influence the rate and pattern of aging of the individual.

In Biological terms, aging represents the molecular, biochemical, physiological and structural changes that take place in an organism following the attainment of reproductive maturity. At all levels of biological organization, there is a progressive decline in adaptation to stress, and in the capacity of the system to maintain the homeostatic balance in the functioning of various organs that is characteristic of the adult organism. In general, life span of a multicellular organism is characterized by a smooth transition from the developmental phase to the reproductive phase. This is followed by a short or long period of senescence (aging) terminating in death. Aging rarely occurs in feral animals that live in the wild as they are eliminated by external hazards and do not live long enough to undergo the aging phenomenon (Hayflick 2000 see below). The species which are, however, protected from external hazards like the humans, domestic cats and dogs, have long periods of senescence. The life span appears as a continuum in the latter because there are apparently no sharp, demarcating changes between the three phases. The rate, pattern and duration of senescence depend on the quality of the adulthood period, and the vigour, vitality and duration of adulthood depend on the quality of

the developmental period. The three phases are inter-related. The adult phase smoothly merges into the senescence phase. Hence the period of senescence should not be considered as an isolated and independent phase of the life span. Information on developmental and adulthood periods may, therefore, help in understanding why we get old and the mechanism of aging.

There is an interesting relationship between the time taken to attain reproductive maturity and maximum longevity among certain species. For example, for man, elephant, chimpanzee, rhesus monkey, cat, rat and mouse the age at maturity is 12, 12, 10, 3, 1.5, 0.25 and 0.2 yrs, respectively, and their maximum life span is 120, 70, 40, 25, 25, 4 and 3 yrs, respectively (Kanungo 1994, Rose 1999).

There are exceptions to this general pattern of life span and aging. Those that live and attain adulthood in the wild have a short duration of senescence due to extrinsic factors as their physical abilities decline after attainment of adulthood, their ability to compete with younger individuals for food declines, and they succumb to starvation, predation and other stresses. Hence aged animals are rarely seen in the wild. Also in the semelparous species like the pacific salmon, marsupial rat of Australia and *Octopus,* the duration of senescence is very short, though for a different reason. They die soon after reproducing only once. It appears that the single act of reproduction so depletes certain vital factors of the organism that it is unable to regain these factors quickly and, therefore, undergoes fast deterioration of activity leading to death. This is also corroborated by the observation that among the mammals, mice and rats which give birth to several progeny and at short intervals have short life span, whereas elephants and human species which generally give birth to only one progeny at a time and at long intervals, have much longer life span. Another important phenomenon is that in the domesticated and protected animals including the human species, cats and dogs, the period of senescence is considerably prolonged during which various types of aging symptoms appear, and the animals suffer from different types of diseases that ultimately lead to their death. It is this period which is the concern of the scientists interested in the problem of aging.

Evolution of Aging

The perpetuation and evolution of a species can occur through two strategies. In one, the organisms produce a large number of offspring, either once or a few times, and then die. Their young fend for themselves, and hence have a high mortality rate. A few, however, survive till reproductive maturity, reproduce, and die soon after. Such animals do not have long lives because if they do, the population would grow exponentially, and beyond a point would crash due to shortage of food, competition among individuals, and problem of space. In the second strategy, the reproductive rate is low and is linked with parental care. The mortality rate is low, and the individuals have long lives during which they reproduce several times, thereby ensuring perpetuation of the species.

Even though natural selection is believed to exert its influence by decreasing the frequency of genes that cause senescence, and increasing the frequency of genes that prevent it, yet senescence occurs. The most widely accepted explanation for evolution of senescence is that it is beyond the influence of natural selection because predation, starvation, disease, and other environmental hazards cause the death of most wild animals before they reach the age at which bodily fitness starts to decline (Medawar 1952, Rose 1985, 1991, Hayflick 2000, Kirkwood & Austad 2000). That is, very few individuals live long enough to age. Since the number of individuals is very small, the force of natural selection is not sufficient to select the genes that cause senescence. It is believed that natural selection is weak at this level of organization. Therefore, it cannot sustain the traits that are detrimental to individuals, even if they otherwise benefit the individuals in some way (Williams 1957). Even so, all animals undergo senescence and do not die immediately after reproducing once or twice, especially when environmental hazards are low. Therefore, increased longevity is likely to have evolved as a consequence of longevity assurance mechanisms (Sacher 1978).

It is possible that senescence is a consequence of the effects of certain genes that are selected for their beneficial effects in adult life when the force of selection is the strongest. These genes are believed to be pleiotropic and have detrimental

effects in later life (Williams 1957). In other words, the senescence phase is a fall out of late acting effect of certain genes that were selected for some benefits to the adult. So there is no senescence causing gene(s). The pleiotropic effects that lead to senescence vary in duration. In species like the salmon, which do not take care of their young and in which the young have a high mortality rate during the interbreeding period, the deleterious effects of the pleiotropic genes are expressed suddenly because natural selection cannot oppose them, and death of females due to senescence occurs within days (Williams 1957). In species such as higher vertebrates that care for their young, the post-reproductive period begins only after the young no longer benefit from parental care. In these animals the deleterious pleiotropic effects of the genes that were selected for certain benefits to the adult, such as reproduction, generally begin to be expressed after the reproductive period is over.

In support of the above, Gustafsson and Part (1990) provide the example of the collard flycatcher (*Ficedula albiocollis*) in which costly reproduction in early life accelerates senescence for fertility under natural conditions. The birds form an isolated population, and apparently are not exposed to environmental hazards in their natural habitat, unlike the birds that share the same ecological niche with other species. Yet their reproductive performance decreases with age, which may be due to some innate deterioration of the individual. A comparison was made between the birds breeding for the first time at the age of 1 year with those that started breeding at the age of 2 years. Then they compared individuals with experimentally enlarged brood size with those having reduced or unmanipulated brood size. The females that started breeding from the second year laid larger clutches throughout their subsequent lives than those that started breeding in the first year. So the former show late breeding and high fecundity, and the latter show early breeding and low fecundity. Furthermore, it was found that females with enlarged brood size laid smaller clutches later in life than those with reduced or unmanipulated broods. Thus, the cost of early reproduction is paid for in the form of reduced reproductive performance in later life. This is believed to be the result of selection for high early fertility during evolution (Williams 1957). This is true for birds that breed in the first year and have a large clutch size.

Thus, it appears that reproduction itself induces senescence for fertility. It is likely that reproduction depletes certain essential factors that are necessary for maintaining reproduction itself, and the animal is unable to replenish these factors quick enough to maintain the fast reproductive rate. It cannot be due to ecological risks because that should affect only survival (Partridge 1989). Moreover, the collared flycatcher lives in an isolated population and, therefore, is subjected to minimal ecological hazards. Thus, the actions of the pleiotropic genes, which are selected for conferring reproductive advantage by enabling early reproduction, have effect on fertility itself later in life.

That such pleiotropic genes influencing senescence may exist has been inferred from breeding experiments in the laboratory. Selection for late reproduction in the female fruit fly increases longevity, decreases early fecundity, and increases late fecundity (Rose 1984). Selection for early reproduction decreases longevity in *Tribolium* (Sokal 1970). If pleiotropic genes have been selected by natural selection to confer benefit to the adult, but have deleterious effects that cause senescence later in life, then manipulation of such genes may extend the life span and prevent or postpone senescence. However, there is the danger that such manipulation may deprive the adult of the beneficial effects of the genes.

If one or more pleiotropic genes acting for the benefit of the adult have deleterious effects later in the life span, then why should the contributions of these genes vary so greatly in different species? Available data, though insufficient, suggest that pleiotropic effects are minimal in birds and are important in large mammals (Nesse 1988). Despite the variations in the period of senescence, the declining force of natural selection with increasing age due to a sharply declining number of individuals may be the basis for the evolution of senescence.

Arking (1991) has analyzed the onset of senescence and longevity in *Drosophila* by generating long-lived strains through artificial selection. Both the mean and the maximum life spans are extended, the mean life span exceeding

the maximum life span of the controls. This is due to a genetically based delay in the onset of senescence. The increase in the duration of the presenescent period is under both genetic and environmental control. Senescence itself is not under genetic control and appears to occur stochastically. Selection for decreased longevity is unsuccessful, which may be due to the requirement of a minimum species-specific life span to attain reproductive ability for the perpetuation of the species.

Not only do genes that confer reproductive ability need to be selected through natural selection for the perpetuation of the species, but also it is likely that genes that control earlier events leading to reproductive maturity have also been selected; otherwise a mature organism will not be produced. However, any gene or genes that would perpetuate the juvenile stage and prevent maturation would be selected against. Thus, each stage of an organism leading to maturity is stabilized by the selection of specific genes, since the force of natural selection up until maturity is high because the number of individuals is high. Natural selection is unable to operate effectively after the attainment of maturity, since the number of individuals is considerably decreased. Hence, even if some of the traits of senescence, such as parental care, are advantageous, the possibility of selection of these traits is low.

Phenotypic Changes during Aging

Several structural, biochemical, physiological and functional changes occur both at the tissue and cellular levels as the animals age. Some of them are: decrease in the number of post-mitotic cells such as neurons and muscle cells, decrease in muscle contractility, decrease in the levels of hormones, antibody and immunocompetence, decrease in permeability of membranes, decrease in the levels of enzymes, though some enzymes do not show any change and a few others increase in level. However, cross-linking and tensile strength of collagen, wrinkles in skin, free radicals, age pigment in neurons, etc. increase after adulthood (see Finch 1990, Schulz-Aellen 1997). These changes are, however, secondary in nature, and do not explain the basic mechanism of aging at the level of genes. For example, increase in free radical level is due to the decrease in the levels of the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GP) and catalase that scavenge the free radicals as they are produced in mitochondria during respiration. The question that arises is why do the levels of these enzymes decline. A plausible reason may be that the expression of the genes that code for these enzymes declines which leads to lower levels of their messenger RNAs (mRNA), and consequently lower levels of the enzymes. Hence the basic or primary cause of the cellular changes mentioned above can be found at the level of genes. Only then it may be possible to manipulate the expression of the genes that code for the enzymes and proteins responsible for the secondary changes. This would defer or delay such changes and extend the adulthood period or postpone the onset of the aging process.

The decrease in the levels of enzymes after adulthood may be due to a decrease in transcription of their genes which would depend on the structure of the chromatin in which the genes are housed. Transcription depends on the compactness of the chromatin. Whether or not the chromatin gets increasingly compact as a function of age, especially in the brain and skeletal muscle which have cells that are post-mitotic and stop dividing soon after birth, was investigated by digesting the chromatin of the brain of rats with the enzyme, DNAse I, that cleaves the DNA at 10 bp intervals. Resolution of the DNA fragments by gel electrophoresis showed that 10 bp and 20 bp fragments are produced in a significantly lower amount from the brain of old rats. This showed that the DNA becomes increasingly compacted with the histones in the chromatin with increasing age. Hence transcription gradually decreases as an animal ages (Chaturvedi & Kanungo 1985). This was supported by the finding that the repetitive DNA sequences in the genome get increasingly methylated with age, especially the CpG doublets in –CCGG- sequences (Rath & Kanungo 1989). It is known that methylation of –CCGG- sequences contributes not only to compaction of chromatin but also repression of genes. The above observation was substantiated by the finding that the histones of the chromatin of old rats is considerably less acetylated and there is a corresponding decrease in transcription of the chromatin. This may be responsible for the lower

expression of genes and lower levels of enzymes in old age (Kanungo & Thakur 1979).

That the basic or primary cause of aging may be found at the level of genes is evident from the following observations: all individuals of a species have more or less a fixed maximum life span or longevity: humans 120 yrs, elephant 70 yrs; horse 60 yrs; cat 25 yrs; rat 3.5 yrs; the insect *Drosophila* 60 days, the free living nematode *Caenorhabditis elegans*, 15 days. The progeny of these species inherit the longevity characteristics from their parents through the genes at the time of fertilization. Likewise, long-lived parents have generally long-lived children and short-lived parents have short-lived children. Identical twins have similar longevity (Abbott et al. 1978). These observations also indicate that the basic cause of aging and longevity may lie at the level of genes. However, though the genes determine longevity of individuals of a species, the differences in longevity found among the individuals of the same species are due to, besides their genetic constitution, the types of nutrition they take and various types of stresses such as radiation, pollution, temperature, etc. including psychological stress to which they are exposed to during their earlier phases of the life span. These factors may also be responsible for the expression of undesirable genes such as oncogenes, and account for the differences in aging and life span of the individuals of the species, and also the pattern and rate of their aging.

Another important observation is that the functions of different organs begin to deteriorate at different times of the life span, and also at different rates. For example, longitudinal studies have shown that in humans, vital capacity of the lung, kidney filtration rate, cardiac index, basal metabolic rate, etc. decline at different rates after age 30 (Shock 1985). Certain specific genes are involved in the functioning of each organ as it performs specific functions, besides some constitutive genes which are common to all organs. Hence deterioration of functions of various organs is likely to be due to deterioration of expression of both types of genes in these organs. There is no single "master" gene that initiates the deterioration of functions of all organs and the whole organism at the same time, because if such a gene were present, the functions of all organs would begin to deteriorate at the same time of the life span and at the same rate. This is not the case.

Different investigators have used different animal models such as rats and mice, *Drosophila*, *C. elegans*, and also fibroblast cells in *in vitro* culture to study the molecular and genetic basis of aging. Each model has provided some useful data, and there is now a fair understanding of the changes that occur in genes as an animal ages, and the methods by which such changes may be prevented.

Mechanism of Aging

Using different models, and biochemical, molecular and genetic data, different investigators have proposed different theories and mechanisms that attempt to explain the primary cause of the aging process at the level of genes. Some of them are: (i) "Somatic mutation" theory (Szilard 1959), (ii) "Free radical" theory (Harman 1957), (iii) "Gene regulation" theory (Kanungo 1975, 1980, 1994). In addition, data on manipulation of specific genes in model organisms, effect of caloric restriction and telomere shortening on aging have provided useful information on the mechanism of aging.

Somatic Mutation Theory

According to this theory mutations that occur randomly and spontaneously damage genes and chromosomes in post-mitotic cells during the life span of an organism and gradually increase its mutation load. This decreases the production of functional proteins leading to a decline in functional ability and aging of the organism (Szilard 1959). Curtis (1963) found that exposure of mice to sublethal doses of X-ray increased chromosomal aberrations in the liver and shortened their longevity. However, the symptoms of aging resulting after X-ray exposure were not similar to those of normal aging. Besides, studies on the wasp, *Habrobracon*, have shown that both males which are haploid and females which are diploid have the same life span (Clark et al. 1963). If DNA damage is the cause of aging, the diploid should live longer as it has two sets of each gene. Also both diploid and tetraploid human fibroblast cells have similar life span in culture (Thompson & Holliday 1978, Holliday & Kirkwood 1981).

Hart and Setlow (1974) reported a positive correlation between maximum life span of a species and its capacity to repair UV-induced DNA damage. However, such relationship was not found by Turner et al. (1982). Accumulation of single strand breaks (Samis et al. 1966) and double strand breaks (Tice & Setlow 1985, Rao et al. 2000) in DNA have been reported during aging. The enzyme, DNA polymerase β, is involved in the repair of DNA damage (Waser et al. 1979, Wilson 1988) and its level decreases with age in the brain of rats (Rao 2000) like several other enzymes that decline with age. Hence it is the decline in DNA polymerase β which is responsible for the decrease in the repair of damaged DNA. It is necessary to know why the level of DNA polymerase β declines, that is, why the expression of the gene for this enzyme declines in order to understand the primary cause of the decline in repair of damaged DNA with increasing age.

Free radicals and Aging

Harman (1957) proposed that free radicals which are highly reactive molecules or atoms accumulate with age and inactivate macromolecules such as DNA, RNA, proteins (enzymes) and lipids, and cause aging. In young age, the enzymes, superoxide dismutase (SOD), glutathione peroxidase (GP) and catalase detoxify the free radicals and act as scavengers of free radicals. Thus they protect the macromolecules from inactivation. The levels of these enzymes decrease with age (Tolmasoff et al. 1980). Also, vitamins C and E detoxify free radicals. Mammalian SOD is a dimer with two copper and two zinc atoms (Cu-Zn-SOD). Superoxide radicals are produced as follows:

$$O_2 + e^- \rightarrow O_2^{\cdot-}$$

The superoxide radical $O_2^{\cdot-}$ is very toxic. It is neutralised by SOD as follows:

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$$

H_2O_2 is less toxic and is neutralized by catalase or GP as follows:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

H_2O_2 and $O_2^{\cdot-}$ may be converted to the very reactive hydroxyl radical (OH^-) which causes extensive damage to DNA, lipid membranes and enzymes. Such damage may contribute to various disease states. According to the free radical theory of aging (Harman 1957) reactive oxygen species (ROS) – associated damage results in cellular demise and tissue degeneration leading to death. Aged animals do accumulate oxidative damage. These damaging effects of ROS are to some extent prevented by antioxidants such as SOD, catalase, GP etc. However, since the levels of these enzymes that are coded by specific genes decrease with age, the ROS are not completely removed and keep on accumulating and damaging the macromolecules and cell membrane. So the basic cause of aging is not the free radicals but the lower levels of enzymes that remove them. Hence it is necessary to find out why the expression of genes that code for the free radical scavenging enzymes decrease.

There is a positive correlation between the specific activity of SOD and life span potential of mammals. That is, mammals with long life spans have higher SOD (Tolmasoff et al. 1980). Also, the activities of SOD and catalase decrease in the liver of the rat with increasing age (Semsai et al. 1989). Reactive oxygen species such as superoxide radical, primarily arise as by-products of normal metabolic activities, especially during oxidative phosphorylation in mitochondria, and during β-oxidation of fatty acids in peroxisomes. They are believed to influence the etiology of age-related diseases (Ames et al. 1993, Beckman & Ames 1998). Extrinsic agents like UV also generate ROS. If ROS contribute to aging, then the aging process may be slowed down when the production of ROS is reduced or the levels of antioxidants within the cells are raised.

Whereas SOD, GP and catalase prevent DNA damage by removing free radicals, proteins like Ku86, DNA ligase, etc. repair the DNA strand breaks which increase with age. Vogel et al. (1999) generated knock-out mice in which Ku86 gene was deleted. The Ku-mutant mice prematurely exhibited age-specific changes characteristic of senescence. This shows that Ku86 dependent DNA repair is important for postponing the aging process.

In normal mice the proto-oncogene of SHC locus codes for $p66^{shc}$ protein. Its tyrosine and serine residues get phosphorylated when exposed to ROS. Migliaccio et al. (1999) generated knock-out mice

lacking the gene for $p66^{shc}$ in which the protein was not produced. The mice lived 40% longer.

A *Drosophila* strain carrying a null mutation in SOD gene has reduced life span (Reveillaud et al. 1991). Also transgenic strains of *Drosophila* which have more than one SOD gene have longer life span. Such *Drosophila* were produced by transfecting their embryos with cDNA of bovine SOD. The flies produced their own SOD as well as the mammalian SOD. Transgenic *Drosophila* that over express SOD and catalase (Orr & Sohal 1994) and human SOD in *Drosophila* motoneurons (Parkes et al. 1998) have extended life span (see review by Finkel & Holbrook 2000). Mutants of *C. elegans, Drosophila* and mice which have extended life span as compared to the wild type are more resistant to oxidative stress. Thus SOD is essential for neutralizing superoxide radical. Several investigators have shown that genetic manipulations of *Drosophila* increase their life span (Parkes et al. 1998, Sun & Tower 1999, Migliaccio et al. 1999).

A genome-wide study using high density DNA microarray representing 7829 genes of *Drosophila* was carried out for whole genome analysis (Zou et al. 2000). Also paraquat, a herbicide that enhances free radical production, was added to the diet to examine its effect on the genes that are involved in scavenging free radicals. Expression of some genes changed with age, but not after paraquat treatment. Hence free radicals may affect transcript (mRNA) levels but such effect is indirect.

Wild type *C. elegans* have a mean life span of 15 days at 20°C. When they are cultured in a medium containing synthetic chemicals (mimetics) that act as antioxidants like SOD/catalase, their life span is increased by nearly 44%. Also, treatment of prematurely aging worms by the mimetics resulted in normalization of their life span (nearly 67% increase). Thus oxidative stress is an important factor that determines life span, and it can be counteracted by pharmacological intervention (Melov et al. 2000).

Genetic studies conducted on various species, *C. elegans, Drosophila* and mice have shown that genetic variants which lower metabolic rate and provide protection against free radicals, also extend longevity. Hence such genetic manipulations that alter global functions such as prevention of the effect of ROS, lowering of metabolism, etc. may be a useful approach to extend the life span of animals. But the basic question: why do the levels of SOD, GP and catalase decrease, as well as the expression of their genes decrease, remains unanswered. If their levels do not decrease it will have a significant positive effect on the life span. Thus endogenous oxidative stress is an important determinant of aging as it causes damage to DNA/genes besides enzymes, etc. but it is not the primary cause of aging.

Genetic Changes

Specific genes and combinations of genes are believed to determine the characteristics of senescence, and the potential life span of organisms (Finch 1990, Finch & Ruvkun 2001), though environmental factors and nutrition influence both the characteristics. During the past decade several genetic studies have been carried out on yeast, *C. elegans, Drosophila*, and mice that suggest that longevity and aging of these organisms have a genetic basis. Long-lived and short-lived mutants of these animals have been produced by exposing them to various types of stress such as higher temperature, free radical producing agents, etc. It has been shown that a long-lived temperature mutant of *C. elegans* has a mutation in its *daf*-2 gene. Also, mutation in *age*-1 gene extends its life span. The sites of mutations in the genes are not known. The *daf*-2 gene codes for an insulin-like receptor which is responsible for glucose transport and metabolism (Strauss 2001). Age-1 gene also codes for another member of the insulin receptor family which may have a similar role. They are similar to those of vertebrates and are membrane bound. Mutations in *daf*-2 gene in *C. elegans* decreases transport of metabolites, enhances stress resistance and increases life span up to 200% (Guarente & Kenyon 2000). In the yeast, *Saccharomyces cerevisiae,* the long-lived mutants have mutations in the *Sch*-9 gene which is also resistant to higher temperature (Fabrizio et al. 2001, Gems 2001). This gene is analogous to the mammalian insulin receptor gene, *InR*, and is, therefore, involved in sugar metabolism (Tatar 2001).

The fruit fly, *Drosophila*, has a life span of 60 days. Its *Chico* gene is homologous to *InR*, and

when mutated extends the life span of homozygotes by about 48%, and heterozygotes by 36% (Clancy et al 2001). Flies with normal *InR* and *Chico* genes have normal life span. So it appears that normal *InR* and *Chico* genes accelerate aging. It is not known how the mutations in these genes extend life span. The long-lived flies with mutations in *Chico* and *InR* genes are, however, sterile. They produce very low levels of juvenile hormone and live longer. Such studies may be useful for biological control of animal pests. When flies are generated by transfecting a normal *Chico* gene into the egg of a *Chico* defective fly, the life span becomes normal. This again shows that normal *InR* and *Chico* genes accelerate aging. Both code for receptors of insulin signaling pathway as in the case of *C. elegans,* and are involved in metabolism. So lowering metabolism may extend life span. This is equivalent to extending life span by caloric restriction. Another gene of *Drosophila, Indy,* codes for a protein that resembles a sodium dicarboxylate cotransporter, a membrane bound protein found in many organisms, from bacteria to humans (Rogina et al. 2000). The protein recycles metabolic products. A mutation in the gene makes metabolism less efficient and doubles the life span of *Drosophila.*

Benzer and his colleagues (Lin et al. 1998) generated mutant *Drosophila* by genetically unleasing one of the insect's transposable elements, a stretch of DNA that can jump around the genome causing mutations wherever it happens to interrupt a gene, and then observed how long they lived. One mutant lived for as long as 100 days (a 35% increase) instead of the usual 50-60 days. The flies were better able to tolerate various types of stress such as higher temperature and starvation, and were more resistant to paraquat, a herbicide that generates free radicals. Hence resistance to stress makes an organism live longer. They named the gene that got mutated resulting in prolongation of the life span, *methuselah (mth).* The gene is named after Methuselah who according to the Bible, lived for 969 years. The *mth* gene codes for a protein that has homology with GTP-binding transmembrane protein receptors. This suggests that the fly may use signal transduction pathways to modulate stress response and life span. The protein is analogous to the *daf-2* gene of *C. elegans* which also codes for a signal pathway receptor protein. Recently, they have studied the 3-D structure of the 195 residue external domain of the MTH protein by X-ray crystallography at 2.3 Å resolution. The topology of this fragment reveals three domains which form a shallow interdomain groove that has a tryptophan which may represent the ligand binding site. Neither the site of the mutation nor the ligands that bind to MTH receptor protein are known (West et al. 2001).

A recent study (Puca et al. 2001) conducted by genome wide scan has identified a locus on human chromosome 4 that confers exceptional longevity in humans. This study was conducted on 308 individuals who belong to families whose members have exceptional longevity, that is, over 90 years. The locus has genes that exert a substantial influence on the ability of the individual to achieve exceptional longevity. This shows that longevity characteristic is inherited. It also explains why there is familial aggregation of exceptional longevity and why longevity runs in families. It is of interest that these individuals who have exceptionally long life span, from 90 to 110 yrs, age rather slowly and somehow do not suffer from old age diseases. Identification of the genes of chromosome 4 and their role in various functions shall provide valuable information for the understanding of the genetic basis of aging.

The genetic studies show that certain genes are responsible for specific functions that affect transport of nutrient across membrane, metabolism and longevity. At what stage of the life span of the normal individual they get triggered and what triggers them need to be elucidated. Also, the aging phenomenon being multifactorial is expected to involve several genes as several organs age at different times of the life span of an organism, and at different rates. So cascades of genes, rather than one gene per organ, are likely to operate to cause aging of an organism. The above approach no doubt will provide further insight into this mechanism.

Gene Regulation Theory

The observation that different organs in an organism (i) begin to age or deteriorate in activity at different times of the life span after completion

of growth period, (ii) their rates of decline are different, (iii) and the decline in the levels of different enzymes is different in different organs during aging led to the concept that aging of an organism and an organ is multigenic and multifactorial. Different organs use different sets of genes for their activity because of the differences in their functions, while some of the genes, especially those required for energy production, are common to all the organs. An organ may start aging after the growth period is complete due to any one or more of the key genes decreasing in expression which may be different for different organs. That is why we all do not age the same way and all organs do not begin to age at the same time of the life span in all individuals of a species. Experimental data on several enzymes have shown that not only the levels of most enzymes decrease, but certain enzymes show no change in activity as a function of age, and a few even increase in activity. Besides, even the isoenzyme patterns of enzymes change suggesting changes in the expression of the genes that code for the subunits (protein chains) of the multimeric enzymes during aging. The following examples support this concept.

The heart and the brain are highly aerobic tissues and require a constant supply of oxygen for energy production through the Krebs cycle to keep them active. In the adult humans, if the oxygen supply to the heart and brain cells through the blood vessels is cut off for 3-4 min due to a clot, the cells die and the organs get damaged. The period of tolerance of these cells to this anaerobic condition gradually decreases with increasing age. In an old person, the organs may get damaged in 1–2 mins. Studies on the rat have shown that this is because there is a gradual decrease in the enzyme, lactate dehydrogenase (LDH), more specifically M_4–LDH isoenzyme. LDH is a tetramer made up of two types of subunits, M and H, assembled in different ratios to form five isoenzymes: M_4, M_3H_1, M_2H_2, M_1H_3 and H_4–LDH. M_4-LDH is more efficient in energy production via glycolysis in anaerobic condition as it converts pyruvic acid to lactic acid faster. So the decrease in M_4- LDH is responsible for higher frequency of damage to heart and brain in old persons (Singh & Kanungo 1968). The M and H subunits are coded by separate genes. Hence the decrease in M_4–LDH is due to lower expression of the M gene. The decrease in M_4-LDH is far greater in the aerobic tissues, heart and brain, than in anaerobic tissues, skeletal muscle and liver. Fibroblast cells kept in anaerobic condition have more M_4-LDH than those kept in normal condition. Therefore, anaerobic condition induces M_4-LDH production. Hence yogic exercises with emphasis on holding the breath for long periods may slow down the decrease in M_4-LDH, and maintain the activities of the brain and heart in anaerobic condition for longer periods. Changes in isoenzyme patterns have also been reported for esterase (Hall 1969), alcohol dehydrogenase (Dunn et al. 1969) and alanine aminotransferase (Patnaik & Kanungo 1976).

Immunological and kinetic studies do not show any changes in the primary structures of enzymes in old age (Kanungo & Gandhi 1972, Srivastava & Kanungo 1979). This suggests that the decrease in the levels of these enzymes is due to the decrease in transcription or expression of their genes, and not due to incorporation of errors or wrong amino acids into the proteins (Orgel 1963) or any structural changes in the proteins.

Another key enzyme, creatine phosphokinase (CPK) required for energy (ATP) production for contraction of heart muscle, decreases in mice after adulthood. In humans, it leaks out to the blood after cardiac infarction and is used for diagnosis of heart attack. The decrease in CPK level is due to the decrease in expression of the CPK gene. This is due to the decrease in the levels of certain nuclear proteins that bind to two repetitive DNA sequences, $(A)_{22}$ and $(GTTT)_8$ and two other sequences, MEF and E boxes, that are located in the promoter region of the gene and regulate its expression. The levels of these proteins can be raised by administration of steroid hormones to mice. Hence the basic cause of the decline in CPK level is that alterations occur in the regulation of expression of the gene due to the decrease in the nuclear proteins that bind to its promoter (Shanti & Kanungo 2001).

Likewise, fibronectin (FNT) is a protein responsible for wound healing, morphogenesis, cell-cell interaction, etc. It is synthesized in the liver. Wounds take longer to heal in old age. This was found to be due to lower level of FNT and lower expression of the FNT gene in the liver of old rats.

The promoter of the FNT gene has several *cis*-acting elements that bind to specific nuclear proteins and are involved in regulation of expression of the gene. One of the *cis*-elements is cAMP-responsive element, CRE, that has an inverted repeat sequence, 5'-TGACGTCA-3'. It binds to three nuclear proteins of the liver, and their levels decrease with age. This may contribute to the lower expression of the gene and lower levels of FNT in old age (Singh & Kanungo 1993).

Birds produce large amounts of proteins, vitellogenin (VTG) in the liver, and ovalbumin in the oviduct, for egg production. VTG is required for egg yolk, and ovalbumin for egg white. Synthesis of these proteins is dependent on steroid hormones. The levels of steroid hormones decrease after adulthood, so does egg production. Using the bird, Japanese quail, as a model it has been shown that the expression of VTG and ovalbumin genes also decreases in the respective organs after the peak egg laying period. These genes have in their promoters specific sequences that bind to specific nuclear proteins whose levels also decrease after the peak period of egg laying. However, if the birds are administered steroid hormones, not only the expression of the two genes, and production of the two proteins increase, but also the levels of the specific nuclear proteins increase (Gupta et al. 1998, Upadhyay et al. 1999). It is of interest that the level of 17 β-estradiol receptor which is known to induce specific proteins decreases in the brain of rats after adulthood (Kanungo et al. 1975). Hence the basic cause of aging is not damage to specific genes but regulatory changes in the expression of genes which can be induced and manipulated by administration of hormones and other effectors.

Induction pattern, however, is different for different enzymes. Acetylcholinesterase, essential for nerve conduction, decreases in the brain of rats after adulthood. However, it can be induced by administration of 17 β-estradiol to the rat and raised to adult level (Moudgil & Kanungo 1973). Induction of tyrosine aminotransferase (TAT) and tryptophan oxygenase (TO) in the liver of rats is similar in young and old rats after administration of dexamethasone. However, under cold stress, induction of TAT is lower in the old, but there is no difference for TO (Wellinger & Guigoz 1986). It may be noted that whereas dexamethasone, together with its receptor, binds to the TAT gene directly to induce it, induction by cold stress involves a complex array of steps. Thus induction of enzymes by hormones or other factors is a selective process and is organ-specific.

Recently it has been shown that expression of several genes is involved in the functioning of an organ, and alterations in their expression may affect its function. Weindruch's group (Lee et al. 2000) has shown that certain genes in the neocortex and cerebellum of mice are differentially expressed as they age, and some of these changes are prevented by caloric restriction (see under caloric restriction). Welle et al. (2000) studied the expression of genes of vastus lateralis muscle of 21-24 yr and 66-77 yr old men by serial analysis of gene expression. Genes for eight enzymes of the glycolytic path, and those of myosin heavy chain 2a, NADH dehydrogenase and cytochrome b are down-regulated in old men.

These studies show that aging is not due to the down regulation, for example, of a few genes like those of LDH in the brain, FNT in the liver of rat, CPK in the heart of mice, VTG in the liver and ovalbumin in the oviduct of birds, and TAT and TO in the liver of rat. Several genes are required for making these different complex organs function, and attempts to delay or postpone the aging process would require manipulation of an array of genes.

The above findings provide support to the "Gene regulation" theory of aging that was proposed by Kanungo (1975) and elaborated in 1980 and 1994 to explain both the cause of aging, and variation of aging and life span of different individuals within a species. It proposes that adulthood of an organism is maintained by homeostatic functioning and maintenance of several organs, each of which, in turn, depends on the expression of several genes. Expression of these genes is modulated by several factors including hormones, proteins, etc that are produced by different organs. Hence there is interaction among the organs, and their functioning is interdependent. Stresses of various kinds encountered during adulthood such as reproduction, nutritional deficiencies, temperature, pollution and radiation,

besides psychological stress lower or raise the levels of these factors. This causes destabilization or breakdown of the homeostatic functioning and maintenance of genes leading to deterioration of functions of organs and the organism. Variations in the pattern of aging and longevity of different individuals within a species are due to different types of stress they encounter, their genetic make up and their adaptability to the various types of stress.

It was further proposed that aging is not programmed like the various stages of development during which specific genes are switched on and switched off in a time bound manner leading to reproductive maturity. Nor is aging a disease. It is caused by the running down or breakdown or destabilization of the homeostatic functioning of an array of genes required for the maintenance of adulthood, as the organism gradually fails to adapt to the external and internal variations of factors that influence its genes. This also determines the period of senescence, as for example, species which live in protected environment and do not encounter environmental hazards like the domestic cat, dog and humans have longer period of senescence than the species which are evolutionarily close, but live in the wild. But eventually death occurs not because all organs stop functioning but because of malfunctioning of one or more organs to such an extent that other organs that are dependent on it are also affected.

It is speculated that the aging phenotype is a consequence of the adaptation that an organism makes to the changing environment to continue to live and reproduce. This is achieved by preventing destabilization of homeostasis of the cellular environment. This has resulted in an extension of both the developmental and reproductive (adulthood) phases. Possibly such a mechanism operated during mammalian evolution that resulted in a gradual increase in their life spans. Fossil records show that the earliest mammal had a life span of about 1.5 yrs, humans now live for 120 yrs. This has occurred possibly due to fine-tuning of the various components needed for these phases by selection processes during evolution to adapt to the changing environmental conditions. This has slowed down destabilization of homeostasis and has extended these phases of the life span, and thereby has increased the longevity. It is hoped that with the methods available it may be possible to further fine-tune and stabilize and maintain the cellular environment and extend the adulthood period.

Caloric Restriction and Aging

It was first reported by McCay et al. (1935) that young rats fed a diet that was nutritionally adequate but low in calories had a slower growth rate, matured later than the rats fed *ad lib* and lived 30-40% longer than controls. Recently, several investigators have shown that expression of certain genes that are down-regulated in the brain and skeletal muscle of old mice are reversed by caloric restriction. Using high density DNA (oligonucleotide) microarrays representing 6347 genes, Weindruch's group (Lee at al 2000) studied the expression of genes in the neocortex and cerebellum of 5-month and 30-month old mice, and also the effect of caloric restriction (CR) on their expression. Stress response genes such as those that code for heat shock factors, and oxidative stress inducible genes are up-regulated in old mice, but CR suppresses them. Of the 32 genes that are up-regulated in the neocortex in old mice, expression of 19 genes is partially prevented by CR. Of the 23 genes that are down-regulated, 17 are partially prevented, but not the remaining ones. In a similar study on skeletal muscle (gastrocnemius) of mice, aging was found to up-regulate stress response genes and down-regulate genes for glycolytic enzymes, and CR prevents such alterations (Lee et al. 1999, Weindruch et al. 2001). Of the 18 genes that are up-regulated, CR prevents up-regulation of 8 genes completely, and 8 partially. Of the 27 genes that are down-regulated, CR prevents 11 completely and 9 partially. It is of interest that most of the genes that are up-regulated or down-regulated in the brain and muscle are tissue-specific, and CR does not have effect on these genes in old age. This supports the "gene regulation" theory (Kanungo 1975) which postulates that different genes are responsible for aging of different organs.

In a similar study using high density oligonucleotide microarray Kayo et al. (2001) studied the expression profile of 7070 genes of

vastus lateralis muscle of 8 yr and 26 yr old rhesus monkeys, and the effect of CR on their expression. Aging resulted in a selective up-regulation of mRNA of genes involved in inflammation and oxidative stress, and down-regulation of genes for oxidative phosphorylation in mitochondria. When middle-aged monkeys were subjected to CR, there was upregulation of genes involved in mitochondrial bioenergetics. Thus induction of oxidative stress induced transcriptional response may be a common feature of aging in muscle.

Since genetic manipulations that lower metabolic rate in *C. elegans* (Guarente & Kenyon 2000) and *Drosophila* (Clancy et al. 2001) increase their life span, and also increase their resistance to oxidative stress, and caloric restriction of rats extends its longevity (McCay et al. 1935), Jiang et al. (2001) studied the expression of genes of the hypothalamus of young and old mice as it is involved in regulation of metabolism. They used high density oligonucleotide microarray representing 11,000 genes. Also, expression of the genes in the cerebral cortex of mice was studied to find out if the expression of the genes is region-specific. Expression of several genes such as for synaptostagmin, apolipoprotein E, protein phosphatase, cAMP-dependent protein kinase, prostaglandin D synthetase, DNA repair protein, etc. are down regulated several fold in the hypothalamus, and 13-fold in the cortex. Expression of cAMP-dependent protein kinase C decreases by more than 3-fold both in the hypothalamus and the cortex. The enzyme is required for synaptic plasticity and memory formation. This may contribute to age-related memory deficits.

On the other hand, expression of certain important genes are up-regulated in these parts of the brain. Many proteases that are essential for regulating neuropeptide metabolism, amyloid precursor protein processing, and neuronal apoptosis are up- regulated in the aged brain. The gene for prolyl-oligopeptidase increases by 11-fold in hypothalamus and 2.7-fold in the cortex. The protease degrades several neuropeptides and hormones, and thereby may accelerate aging of the brain. Inhibitors of this enzyme have been shown to prevent deposition of β-amyloid in the hypothalamus. Overall, probing with 11,000 genes showed that expression of 99 genes alters in the hypothalamus and 98 in the cortex. Alterations in only 20 genes are common for both the regions of the brain (Jiang et al. 2001). These studies show that expression of a large number of genes undergo alterations which may contribute to the declining brain function as animal ages. It is not known why such changes occur. Only when the mechanism of their regulation is understood, it may be possible to prevent such changes and possibly prevent or slow down the deterioration of brain function.

These studies show not only that changes in expression of genes during aging are tissue-specific, but also that in each tissue expression of several genes is altered during aging as envisaged in the "gene regulation" theory. Studies using DNA microarray, however, do not throw light on why the expression of genes changes, as only expression of genes was studied without finding out why the expression changes. It is necessary to find out the mechanism involved in the alterations in their expression to gain deeper understanding of the mechanism of aging. Moreover, alterations of gene expression by diet does not throw light on which component of the diet is effective, and at which cellular level the effect is produced. Hence even though diet may influence the rate of aging and the life span, it is not the basic cause of aging, nor does it explain the mechanism of aging.

The few genes studied so far: FNT, CPK, VTG and ovalbumin, clearly demonstrate that the changes in their expression during aging are due to changes in some of the nuclear proteins that bind to their promoters and regulate their expression. The point of interest is that the levels of these nuclear proteins can be altered/manipulated by hormones, and thereby the expression of the genes and functions of organs can be manipulated. If inducers that bring about global changes in the expression of genes can be developed, it may be possible to manipulate gene expression and organ function, and postpone or slow down the aging process.

In Vitro Aging

Studies on cells grown in *in vitro* culture have provided useful information on the mechanism of aging. Hayflick (1965) first showed that human

diploid fibroblast cells (HDF) undergo a limited number of divisions in *in vitro* culture before their proliferation gradually slows down and ceases. This cellular senescence is a dominant phenotype as fusion of senescent cells or microinjection of their mRNA into proliferating HDFs inhibits proliferation of latter cells. The relevance of this phenomenon of senescence to aging of organism is supported by the observation that there is an inverse relationship between the average number of mean population doublings (MPDs) that the cells undergo before senescence and the age of donor from which they are derived (Bierman 1978). Also, the maximum number of MPDs that cells of various species undergo before becoming senescent is directly related to the average life span of the species (Rohme 1981). Furthermore, HDFs from individuals with premature aging syndromes such as progeria and Werner's syndrome also display premature senescence in culture.

Cellular senescence superficially resembles quiescence induced by serum starvation or contact inhibition. The cells become locked in G1- like state referred to as Gs. This is different from Go state of normal cell cycle in that senescent cells lose the ability to initiate cell cycle entry under mitogenic stimulation. Interestingly, the sub-nuclear organization of quiescent/senescent HDF is different from that of proliferating cells. In the former, a gene poor chromosome moves from the nuclear periphery to a more internal location. If the cells are made to proliferate, the chromosomes move back to original site (Bridger et al. 2000). Cellular senescence appears to be a mechanism for restricting the growth of the organism and production of tumors, because it is seen that the genetic events that confer an infinite life span (immortal) phenotype to cells also increase their susceptibility to tumorigenic transformation. The malignant tumor cells generally have an immortal phenotype. These observations suggest that the finite life span phenotype is under genetic control.

Cell fusion studies involving normal and immortal human cells have shown that hybrids have limited life spans (Pereira–Smith & Smith 1983, Sager 1989). This shows that the phenotype of senescence is dominant, and immortality results from recessive changes in growth regulatory genes of the normal cells. This is supported by the finding that in a heterokaryon cell hybrid formed by having nuclei of one old and one young HDF in a single cytoplasm, initiation of DNA synthesis in the young nucleus derived from actively proliferating young HDF is inhibited. However, its ongoing DNA synthesis is not inhibited (Burmer et al. 1982). If the senescent cells are treated with inhibitors of protein synthesis before fusing them with young cells, inhibition of DNA synthesis in young nuclei is prevented. Furthermore, when poly-A^+ RNA from senescent HDF is microinjected into proliferating HDF, DNA synthesis is inhibited (Lumpkin et al. 1986). These studies suggest that inhibition of DNA synthesis in young cells is due to proteins, that cellular senescence is dominant, and that immortality of cells is due to recessive changes in growth inhibitory genes. This may be because immortalization requires the loss of both the alleles of a dominant gene.

Investigations to identify the chromosome that is involved in senescence have been carried out by microcell fusion in which single human chromosomes are introduced into immortal human cell lines. Human chromosome 1 induces senescence in an immortal hamster cell line (Sugawara et al. 1990), whereas human chromosome 4 limits the proliferative life span of three immortal human cell lines (Ning et al. 1991). In cell hybrids formed between HDF and immortal Syrian hamster cell line, most cells exhibit limited life span comparable to that of HDF indicating that cellular senescence is dominant in these hybrids. The hybrid clones that do not senesce do not have both the copies of human chromosome I, whereas all other chromosomes are present in at least some of the immortal hybrids. Introduction of a single copy of chromosome 1 to the immortal hamster cell by microcell fusion causes typical signs of cellular senescence, but transfer of chromosome 11 has no effect on the growth of the hybrids. These data indicate that in human, chromosome I may have a role in cellular senescence (Pereira-Smith & Smith 1988).

Oligonucleotide microarray has been used for analysis of expression of genes in various types of cells in *in vitro* culture such as dermal fibroblasts, retinal pigment epithelial cells and vascular

endothelial cells. Strong inflammatory type response is seen in late passage fibroblast cells, but the patterns of expression in the three types of cells differ in their senescent stages. In *in vitro* senescence, mRNA expression patterns vary widely with cell type (Shelton et al. 1999).

The studies on HDF *in vitro* have provided useful insight into two specific events of the life span of cells: (i) senescence of cells is a dominant character, and (ii) the proliferative ability of HDF is controlled by a gene(s) located in chromosome 4. However, cessation of cell division is not senescence, because in higher mammals the neurons and striated muscle cells stop dividing very early in development, then differentiate to perform specific normal functions for several years. Moreover, the type of senescence the HDFs undergo *in vitro*, the biochemical and molecular changes that occur in them after they cease proliferating are not similar to changes that occur in them and other types of cells *in vivo*, because the cells of each organ are influenced by secretions of other organs and therefore, constantly interact with other organs. This physiological state is not provided in *in vitro* culture. Later studies have shown that even though the replicative life span of freshly explanted cells in culture is relatively brief, these cells, *in vivo*, replicate throughout the life of the organism (Rubin 1998, 2002). Even so, the *in vitro* studies have provided useful information on several biochemical and genetic changes that the cells undergo as they senesce. Use of appropriate markers to study senescence of cells *in vivo* would provide useful insight into the mechanism of cellular aging of an organism *in vivo*.

Telomere and Aging

Telomeres are specialized DNA-protein complexes that are present at the ends of linear eukaryotic chromosomes and are important for chromosome stability. They are composed of hundreds of tandem repeats of simple, short and G-rich DNA, and proteins. The G-rich DNA repeats do not code for any proteins and are species-specific. The repeats are TTAGGG in humans and mice, TTAGG in silk moth, TTAGGC in *Ascaris*, $T(G)_{2-3}(TG)_{1-6}$ in yeast and TTGGGG in *Tetrahymena*. The G-rich strand is orientated in 5′ → 3′ direction towards the chromosome terminus. In human fibroblasts, the telomere length is 2-3 kb and in mice ~10kb.The presence of telomeres at the chromosome ends prevents degradation of DNA by nucleases from either end, and protects the genes present farther inwards in the chromosomes from degradation. Also, it prevents the chromosomes from joining with each other at their ends. Besides, it enables the ends of chromosomes to be completely replicated (Blackburn et al. 1997, Evans 2001, Hathcock et al. 2002).

Hayflick and Moorhead (1961) found that human fibroblast cells taken from the new born divide 50 ±10 times when cultured *in vitro*, and then senesce and die. Olovnikov (1973) then found that the chromosomes get shortened in length after each cell cycle. He proposed that this is due to the inability of DNA polymerase to copy the lagging template strand of DNA up to its 3′ end. So the 3′ end of the lagging strand remains unpaired and gets degraded. This was called "end replication problem".

In humans, approximately 65bp of telomeric DNA are lost per cell cycle in dividing cells *in vitro* and *in vivo*, but the enzyme, telomerase, replaces a part of the lost telomere (Blackburn 1994, Blackburn et al. 1997, Greider 1996,1999, Harley 1997, Harley et al. 1990). Most normal human somatic cells lack significant telomerase activity. Hence there is a gradual telomere loss with increasing age. This may contribute to cell senescence and age-related diseases. The loss of telomeric DNA from chromosomes is examined by digesting the nuclear DNA by a restriction enzyme that cleaves the DNA proximal to the telomere, and then carrying out Southern hybridization using labelled telomere as probe. The terminal restriction fragments (TRF) obtained get gradually shorter with increasing age indicating a linear loss of telomeric DNA.

Telomerase is a telomere specific reverse transcriptase (TERT). It is a ribo-nucleoprotein having a repetitive RNA, that varies in length and sequence depending on the species. In humans, the RNA is 450 nucleotides long whose 5′ end, 5′ - cuaaCCCUAAc - 3′, is the reverse complement of the human telomere repeat sequence 3′ – GGGATT – 5′. The RNA serves as template for synthesis of

the repetitive telomeric DNA. It is essential for the telomerase activity. Its catalytic activity is carried out by a protein component. During replication of chromosomal DNA, a primer RNA (~10 nucleotide long) binds to the 3′ end of the leading strand of the duplex DNA in 5′→ 3′ direction to initiate its replication. The other DNA strand (lagging strand) with the 3′ end cannot be replicated as it cannot accommodate an Okazaki fragment. So the parental lagging strand with 3′ end remains as an overhang and is prone to degradation. The "end replication problem" is solved by telomerase which by using its RNA as template replicates the 3′ end segment of the telomeric DNA of the lagging strand. The possibility of the telomere getting indefinitely extended is prevented by a telomere binding protein(TBP).

There is an apparent relationship between telomerase activity, telomere length and aging. In non-dividing and differentiated somatic cells telomerase activity is negligible and telomere is also negligible. In dividing cells telomere is long and telomerase activity is high. Germline cells and human tumour cells have high telomerase activity and long telomeres. However, in knock-out mice in which telomerase is completely inactivated, tumour growth still takes place (Greider 1998). *Drosophila* has no telomere. Its function is taken over by transposon which has no repeat sequences. Persons suffering from Hutchinson-Gilford progeria show accelerated aging syndrome. Their fibroblasts in *in vitro* culture have shorter telomeres than those of age-matched normal cells. In Werner syndrome, the division capacity of fibroblasts is lower, and telomere loss is accelerated (Harely 1997). Somatic cells taken from aged mice do not show significant telomerase loss, and replicative senescence of cells does not play a significant role in aging of most rodent tissues. Hence in short-lived mammals, telomere loss may not be an important mechanism of aging unlike in humans. Further *in vivo* studies are necessary to examine the status of telomere of various tissues of different mammals because *in vitro* studies are not physiologically comparable to *in vivo* conditions.

Though a relationship between telomere loss and senescence has been shown for some types of human somatic cells grown *in vitro*, recent studies do not support this view (Rubin 2002). The stem cells of somatic tissues have long telomere and high amount of telomerase, and they continue to divide throughout the life span. Though the differentiated cells of the tissue have much shorter telomere it is sufficient for the few replications they undergo after differentiation. So telomerase is not necessary for differentiated cells. The likelihood that tumours usually arise from stem cells and show loss of differentiation is consistent with the continued presence of telomerase activity in tumours. Further *in vivo* studies using different organisms and tissues are necessary for an insight into this relationship. Furthermore, how the shortening of the length of telomere influences the expression of genes located throughout the chromosome length is not clear. Nor is it known why the expression of the telomerase gene and the level of telomerase decrease. So though telomere is a unique segment of chromosome, it does not seem to be important for aging of organisms, in general.

Conclusions

On the basis of the above biochemical, molecular and genetic data on the changes that occur during aging in various model organisms such as rat, mice, *Drosophila*, *C.elegans* and *S.cervisiae* it may be concluded that:

(i) No specific gene has been selected during evolution by natural selection to cause aging of an organism. Neither does aging occur according to a set programme. The period between fertilization until attainment of reproductive maturity (adulthood), however, occurs according to a set programme that depends on the sequential activation and repression of an array of genes in a time bound manner. Continuation of the adulthood period depends on the homeostatic maintenance and functioning of these genes by regulatory processes.

(ii) However, adulthood period does not continue indefinitely, and aging of the organism occurs after attainment of adulthood because stresses of various kinds that the organism encounters during its normal adult life affect the expression of genes required for adulthood function, and

destabilize the homeostatic functioning of various organs. This causes a gradual decline in their activity leading to aging.

(iii) This is supported by the finding that mutation of specific genes in various organisms, mice, *Drosophila,* nematode and yeast that affect their metabolism also affect their longevity.

(iv) Studies during the past decade have shown that the decrease in expression of genes is due to alterations in the regulation of expression of genes that are mediated through the promoter regions of genes. Specific nuclear proteins bind to specific sequences of the promoter of a gene, and either accelerate or decelerate transcription of mRNA from the gene. Using genetic engineering techniques it has been shown that the levels of some of the nuclear proteins that regulate the expression of genes decrease after adulthood and their levels can be brought back to adult level.

(v) It is of interest that the decrease in the expression of certain genes that occur after adulthood can be prevented, as for example, (i) expression of several genes by caloric restriction, and (ii) levels of nuclear proteins by steroid hormones. These and other approaches at the level of genes may be used to unravel the mechanism of aging that may help in extending the adulthood period and defer the onset of aging.

Epilogue

As has been mentioned at page 2, increase in human longevity should be accompanied by improved quality of life of the elderly. Longevity may be increased by genetic manipulations; however, the quality of life in old age depends not only on the genetic make up of the individual but also on how he/she is accepted/treated by the family, the society and the government. Biologically speaking, no great changes occur in the individual soon after he retires from the job. But for him the social and psychological changes are drastic and immense. Even though there is no specifically timed event to cause aging, and aging is not programmed, the government/ society has imposed an artificial well-timed programmed event on its own people, that is, making people retire at 60 or 65. This is despite the fact that the persons have acquired in specific jobs the skills, expertise, and experience that are not found in younger individuals. In fact, if early adulthood has vigour, vitality and brilliance, then old age has expertise, experience and wisdom. An old person is a rich resource centre, and should be tapped for service as long as possible. This is the healthiest way to improve the quality of life of the elderly in their twilight years.

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