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Alterations in gene expression during senescence

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Abstract. Data on the kinetics, peptide maps, induction and isoenzyme pattern of enzymes of rats show that functional changes in genes lead to aging in eukaryotes. Covalent modifications, such as acetylation and phosphorylation, of chromosomal proteins which are complexed with eukaryotic DNA to form the chromatin, decrease with age. Digestion of the chromatin DNA by the endonucleases, DNase I that cuts DNA at 10 base pair intervals and micrococcal nuclease (MCN) that cuts the linker DNA, were carried out to probe the conformational changes in chromatin. Whereas digestion by DNase I significantly decreases with age, the digestion by MCN does not. Thus the chromatin undergoes increasing compaction in non-dividing cells resulting in alterations in its fine structure. This leads to decreasing gene expression and progressive senescence.

Keywords. Brain; chromatin; DNase I; micrococcal nuclease.

It is by now well established that several structural and functional changes occur at the organ, cellular and subcellular levels as organisms age. They are: decrease in the number of post-mitotic cells, levels of enzymes, hormones and antibody, permeability of cell membranes, increases in the age pigment, cross-linking and tensile strength of collagen. These changes, being secondary in nature, do not explain the basic cause of aging.

The fact that (a) all individuals of a species have a more or less fixed life-span, (b) a similar pattern of decline of various functions occurs in all animals after attainment of reproductive maturity, (c) the progeny of longlived parents has long life-span and the progeny of shortlived parents has short life span and (d) the life spans of identical twins are the same, indicate that the primary cause of aging may be genetic in nature. However, factors like nutrition, heredity, and stresses such as temperature, radiation, and socio-psychological, etc may account for the variability in the rate of aging and longevity among the individuals of a species.

Two types of changes are likely to occur at the level of genes during aging: (i) The genes may undergo structural changes with passage of time resulting in the synthesis of wrong or undesirable enzymes (proteins) that may cause deterioration of function of the organism. (ii) The degree of expression of genes may change with age causing alterations in the levels of enzymes and deterioration of function. Since each type of enzyme is the phenotypic expression of a gene, various aspects of enzymes have been studied to get some insight into the types of changes that may occur in the genes as an animal ages.

Extensive studies on the activities of various enzymes of the brain, heart, liver, kidney and skeletal muscle of the rat have shown that the levels of a large number of enzymes decrease with age, as for example, lactate dehydrogenase (LDH), pyruvate kinase (PK), cholineacetyl transferase (CAT), alanine aminotransferase (AAT), tyrosine aminotransferase (TAT), carbonic anhydrase, acetylcholinesterase (AChE) and ATPase, (Singh and Kanungo 1968; Moudgil and Kanungo 1973; Koul and Kanungo 1975;Chainy and

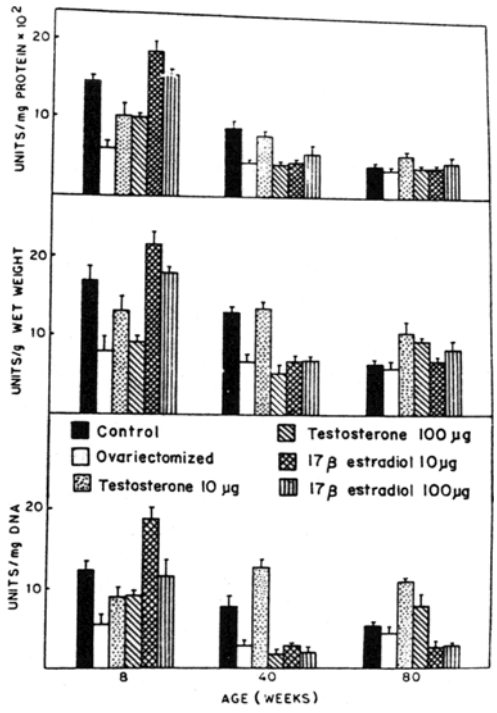

Figure 1. Effects of testosterone and 17β-estradiol on cholineacetyltransferase of the cerebral hemisphere of female rats of various ages.

Kanungo 1978; Kanungo and Patnaik 1975; Ratha and Kanungo 1977; Srivastava and Kanungo 1979). The levels of certain enzymes, such as glutamine synthetase (GS) (Rao and Kanungo 1972), do not change with age, whereas those of cytoplasmic and mitochondrial malate dehydrogenase (MDH) increase with age (Kanungo and Gandhi 1972).

It is possible to reverse the changes that occur in the levels of several enzymes during aging. The levels of certain enzymes which decrease with aging can be raised and brought back to adult levels by administration of steroid hormones, as for example, CAT (figure 1; James and Kanungo 1978), PK (Chainy and Kanungo 1978) and TAT (Ratha and Kanungo 1977). The steroid hormones produce their effects by first binding to a cytoplasmic protein-receptor. The hormone-receptor then binds to specific sites in the chromatin and stimulates the expression of specific genes. A decrease in the level of the steroid hormone may result in a decrease in the expression of the genes. Kanungo *et al* (1975) have shown that the level and affinity of estradiol receptor decrease in the brain of rats with increasing age.

Studies on the kinetics of AChE, PK, GS, ATPase, AAT and TAT, and antigenic properties of purified MDH, AChE and arginase of young and old rats as studied by immunodiffusion do not show any differences between the proteins of the two ages. Also, the peptide maps of myosin and actin of young and old rat are the same. So the primary structure of a protein of an old animal is the same as that of the young. Hence the gene (DNA) coding for the protein does not undergo any change in its primary structure during aging.

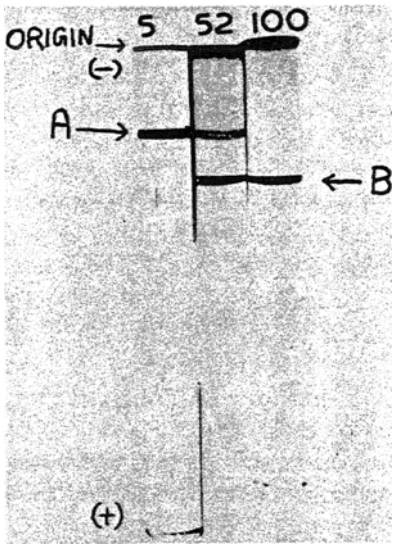

Figure 2. Polyacrylamide gel electrophoresis of soluble alanine aminotransferase of the liver of immature (5-), adult (52-) and old (100-week) female rats. **A.** sAAT-A; **B.** sAAT-B (Kanungo and Patnaik 1975).

Another significant finding is that the isoenzymes of several enzymes, for example, AAT (figure 2; Kanungo and Patnaik 1975), alcohol dehydrogenase and hexose-*P*-isomerase (Hall 1969) show sequential appearance and disappearance as an animal ages. Different subunits of these multimeric enzymes are coded by different genes, and these genes get expressed sequentially during aging resulting in the sequential phenotypic expression of the proteins. This may be due to the modulatory changes in the genes brought about by certain factors that may appear or disappear at specific stages of the life span.

The above data show that alterations that occur in the levels of enzymes may only be due to alterations in the expression of their genes and are not due to any structural changes in their genes. Since the genes are complexed with chromosomal proteins, histones and non-histone chromosomal (NHC) proteins, their expression may be modulated by various types of chemical modifications that occur in these proteins such as phosphorylation, acetylation, ADP-ribosylation and methylation. The first three modifications decrease the net positive charge of the proteins, whereas the last one increases the net positive charge. These alterations in the charge may cause changes in their binding to the DNA which has negative charges. Such changes may modulate the expression of genes by making them accessible or not to RNA polymerase to carry out transcription. Hence studies on these modifications and their effects on transcription were carried out as a function of age of rats. Some of the important findings are given below.

Acetylation of histones has been correlated with transcription in several instances. Incorporation of ^{14}C-acetate into histones decreases with increasing age. Concomitantly, the incorporation of ^{3}H-UMP into RNA also decreases thus showing that transcription decreases as acetylation decreases (Kanungo and Thakur 1979).

Sodium butyrate inhibits deacetylase and thereby hyperacetylates histones. In our

studies, hyperacetylation of histones by Na-butyrate causes stimulation of transcription in the young. The degree of hyperacetylation is lower in the old. The degree of transcription is also lower. 17β-estradiol also causes hyperacetylation of histones and stimulates transcription in isolated nuclei of the brain of young and old rats, but this stimulatory effect of estradiol is not observed in the old.

These findings strongly suggest that conformational changes may occur in the chromatin (a complex of DNA and chromosomal proteins) with increasing age of an organism, particularly in post-mitotic cells like those of the brain and skeletal muscle which do not divide after birth. This may be the reason why the sites on histones and DNA become less accessible for acetylation and transcription, respectively.

If the above hypothesis is correct, then the digestibility of DNA in the chromatin by endonucleases should decrease with age. DNA is wound around an octamer of core histones, H2A, H2B, H3 and H4, to form bead-like structures called nucleosomes. The nucleosomes are connected to each other by linker DNA. This 'beads on a string' structure is the basic structure of chromatin which undergoes coiling due to the binding of histone H1 in the internucleosomal region. Further coiling and compaction of this structure is brought about by NHC proteins.

Two endonucleases, deoxyribonuclease I (DNase I) and micrococcal nuclease (MCN) cut DNA of the chromatin at specific sites and hence were used as probes for studying conformational changes in the chromatin during aging. DNase I cuts DNA at intervals of 10 base pairs (BP) and its multiples, whereas MCN cuts the linker DNA and produces 200 BP fragments and their multiples. The digestion of chromatin of the brain of young, adult and old rats shows that:

(a) The digestibility of chromatin DNA by DNase I decreases with age as seen by both the kinetics of digestion and analysis of 10 BP fragments and its multiples by denaturing polyacrylamide slab gel electrophoresis. In old rats, 10 and 20 BP fragments are far less than in young rats (figure 3). Sodium butyrate, which hyperacetylates histones, causes an increase in the digestion of DNA by DNase I in the young. This effect is greatly reduced in the old. (b) The digestibility of DNA by MCN does not show any change with age as studied by the kinetics of digestion and analysis of 200 BP fragments of DNA by agarose slab gel electrophoresis. Butyrate stimulates digestion of DNA by MCN equally in all ages.

The differences in the digestion by two endonucleases may be due to the differences in the molecular weights and sizes of the two enzymes. DNase I is a larger molecule and the decrease in its digestive activity may be because the closely spaced sites on DNA at 10 BP intervals may become less accessible to it as the chromatin undergoes greater compaction and conformational changes with increasing age. MCN, on the other hand, is a smaller molecule and may reach its sites of action on DNA which are far apart (200 BP) and hence are more accessible to it (Chaturvedi and Kanungo 1983).

These findings show that in the post-mitotic cells such as neurons and skeletal muscle cells in which the age-related functional decline is more pronounced than in the dividing or pre-mitotic cells like those of the liver and bone marrow, the chromatin may undergo increasing compaction with the passage of time as DNA synthesis does not occur. Factors such as steroid and peptide hormones and other effectors that are produced at specific stages of the life span during growth and reproductive maturation may be unable to produce the same effects on the expression of genes as they did in earlier stages. This may cause a gradual decrease in the expression of genes leading to alterations in the levels of enzymes and deterioration of functions.

The above findings support the model for aging proposed by Kanungo (1975).

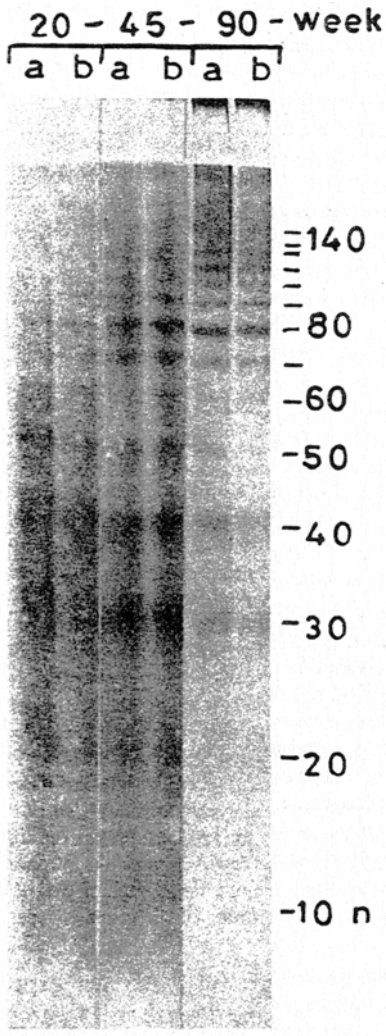

Figure 3. Polyacrylamide slab gel electrophoresis of DNase I digests of (a) nuclei and (b) chromatin of the brain of 20, 45 and 90-week old female rats (Chaturvedi and Kanungo 1983). Electrophoresis was carried out in 10% denaturing urea-polyacrylamide gel. (*n*, nucleotides).

According to this model the regulatory mechanisms necessary for the normal functioning of a set of genes required for various functions including reproduction during adulthood gradually get destabilized as the accessibility of genes gradually changes due to conformational changes in chromatin and the levels of factors that are necessary for induction/repression of these genes are thereby altered. As a result of reproduction and other functions associated with growth, certain factors get depleted which the organism is unable to replenish, and certain other factors accumulate which it is unable to get rid of. Hence there is a gradual decline in the homeostatic functioning of the genes required for the maintenance of adulthood. This leads to deterioration of function and aging. Also, as a result of accumulation of certain factors and depletion of

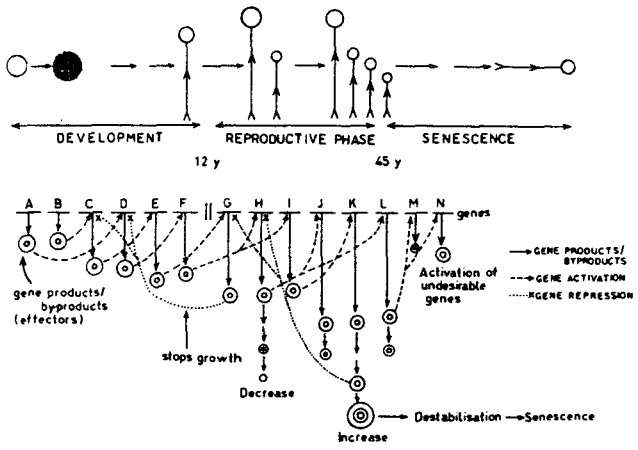

Figure 4. A model for ageing (Kanungo 1980). Upper part—figurative representation of the various phases of the life span, development, reproduction and senescence. Lower part—the number of active genes have been kept at a minimum and the genes that are permanently repressed are not shown for the sake of clarity. Developmental and reproductive phases are dependent on unique genes, A-F and G-L, respectively. No specific genes for senescence are envisaged in this model. Development occurs by the sequential activation of genes A-F, the product of gene A switching on gene B and so on. Some of the genes of the late developmental phase, E and F, switch on some unique genes G and H, belonging to the early reproductive phase. These genes, in turn, switch on sequentially other genes of the reproductive phase. The organism attains reproductive ability when required amounts of gene products are formed. Continued reproduction may cause depletion of certain factors which may be necessary for keeping certain essential genes active. Switching off of these genes may lead to deterioration of certain functions. Continued reproduction may also lead to accumulation of certain gene products (factors) beyond a certain level resulting in the activation of some undesirable genes, M and N, whose products may cause diseases like autoimmune diseases. Thus the decline in physiological functions that begins after a certain period of the reproductive phase may be due to destabilization of the functioning of the genes of reproductive phase or adulthood.

others, certain undesirable and harmful genes get expressed. This may cause the appearance of cancer and autoimmune diseases whose frequency is known to increase with increasing age (figure 4).

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