INTIMATIONS OF MORTALITY

RAMESH SHARMA

FROM even a cursory review, it emerges clearly that there are many theories of ageing. Until now, no single theory has been able to account for all changes although many have attempted to explain, at least, some of the major and most frequent ageing phenomena. Thus, a most productive route to understand the biology and pathology of ageing (including the major age-related diseases of humans) will be one that derives from a combination of molecular, cellular and systemic approaches.

Molecular theories mainly assume that the life-span of any species is governed by the genes (hereditary units) interacting with many environmental factors. Genetic information stored in the genes is transcribed into RNA and is subsequently translated into proteins. These proteins, either structural (collagen and keratin) or functional (enzymes and receptors), govern the form and function of organisms. Ageing may result from changes in DNA template activity which regulates the formation of the final cellular products.

Cellular theories include changes in cellular proteins (structural and functional) and other biomolecules like carbohydrates and lipids that may occur as a function of age. These changes are produced with ageing under the influence of environmental factors like nutrition and stress; they may be chemical and/or structural and involve enzymes, hormones, age pigments, membrane permeability, macromolecule cross-linkage and changes in various cell organelles such as lysosomes and mitochondria.

Systemic theories ascribe ageing of the entire organism to decrease in the function of a key system, such as nervous, endocrine and immune systems. Such decrements could be genetically programmed, as are the early developmental phases of the life-span, or be the consequences of environmental insults. Alterations in the key system will generate changes throughout the entire organism.

There are no less than 11 theories of ageing. Each has its proponents and detractors. This is hardly surprising; because mechanisms that result in ageing, operate at the cell, tissue, organ and body levels. For that reason, ageing is probably multi-causal. Yet, no one has said the last word in bio-gerontology. And there is no denying the possibility that all the theories could be completely wrong and scientists may come up with something entirely unexpected

Molecular theories

Molecular theories originate with the concept that all individuals within a species have a similar life-span and that different species have different life-spans. For example, mayflies live only one day; houseflies 30 days; rats 3 years; dogs 12 years; horses 25 years and humans 70 years. It is presumed that there is some genetic program which determines the maximum life-span for each species. Other arguments for a genetic basis of ageing is that the offsprings of long-lived parents have a longer life-span than those from average-lived parents.

An equally significant contribution to a genetic basis of ageing is derived from the duration of the three phases of the life-span—developmental, reproductive and senescent. In most animals, the reproductive phase occupies a very significant period in the life-span followed by a post-reproductive phase. In mammals, the time taken to reach reproductive maturity is correlated with the maximum life-span. Humans and other long-lived mammals take a longer time to reach reproductive maturity and have in general a longer life-span. The expression of the genetic program which regulates the life-span may be modified by various environmental factors. Some of the major molecular theories involve changes in the genetic program and are mentioned hereon.

Codon restriction

All the genetic information stored in DNA (deoxyribonucleic acid) directs the structure and function of the organism although only part of the total DNA information is utilized by the cell at a given time. The information is transferred from DNA to messenger ribonucleic acid (mRNA) by the process of transcription. The functional mRNA in eukaryotic cells is derived from excision of intervening (non-coding) nucleotide sequences (introns) that are transcribed along and between information sequences (exons) by splicing. This mRNA is then translated into protein. The codon restriction theory of ageing is based on the hypothesis that the accuracy of translation, which depends on the cell ability to decode the triplet codons (3

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bases) in mRNA molecules, is impaired with ageing.

Despite a number of supportive observations, this theory needs further validation. It is still difficult to explain the basic cause(s) for changes in these message-reading molecules and the implications of such changes in ageing phenomena.

Somatic mutation

Alterations in the structure of the DNA molecule change the genetic message and result in differences in protein structure which lead to physiological deficits. This proposed theory was based on the report that rats exposed to limited radiation died at younger age than non-irradiated controls. Acording to this theory, exposure to radiation damages DNA with subsequent addition of mutations which, in turn, lead to progressive loss of genes in non-dividing cells throughout the life-span. The increased rate of mutation and loss of functional genes decrease the production of functional proteins and cause cell death at a critical level. Support for this theory was provided by the observation that limited exposure to X-rays shortens life expectancy and increases chromosomal aberrations (abnormalities) in parallel with increasing doses of X-rays. Older animals have a greater number of chromosomal aberrations than younger. The rate of development of aberrations is more rapid in short-lived mice than in long-lived ones. These observations suggest that natural radiation may also affect the ageing process. However, contrasting evidence negates the role of somatic mutation in ageing.

In some species, such as humans, the sex chromosomes of females are similar (XX) but those of males are different (XY), while in other species it is the reverse. If radiation is a cause for ageing then one might expect a longer life-span for individuals with identical sex chromosomes. However, in most species, the females generally live longer than males, irrespective of the chromosomal composition. Furthermore, chemical substances which change DNA structure have no accountable effect on the life-span of cells in culture. Somatic mutations are no longer regarded as a probable cause of ageing because the rate at which they occur in natural conditions is too low to account for overall age changes. Moreover, most cells have mechanisms for the repair of damaged DNA molecules. These repair mechanisms do not change markedly in aged animals, rather these repair mechanisms appear more effective in long-lived species as compared to short-lived. The presence of multiple copies of the same message coded within the DNA would offer protection to DNA damage. The number of repetitive genes for the major ribosomal RNA is 5-10 in bacteria, 100-300 in drosophila and 250-600 in vertebrates suggesting a correlation between the number of repetitive genes over the life-span of the species. Considering the strong protective device for DNA damage, radiation does not seem to play a major role in accelerating the ageing process nor in causing ageing.

Each phase of life-span has a characteristic duration, rate and rergulatory mechanism

Error theory

The form and function of organisms are governed by specific structural and functional proteins. Certain protein molecules, such as RNA polymerase and aminoacyl-tRNA synthetases, are involved in the production of other proteins. Medvedev first advanced the concept that errors in the transmission of information through RNA to proteins may be responsible for cellular ageing. Errors such as the incorporation of wrong nucleotides into messenger RNA during transcription may change the triplet codons, or incorporation of wrong amino acids into protein during translation may change the amino acid sequence. Orgel further argued that production of functional proteins such as enzymes depends not only on the genetic information stored in DNA but also on the protein synthetic machinery and pointed out that inaccuracy occurs both in protein and DNA syntheses. The initial error in proteins may be low but it increases exponentially with the passage of time and may lead to an 'error catastrophe' and ultimately death of the cell. The hypothesis creditably stimulated a great deal of work, both theoretical and practical, to demonstrate whether or not old organisms would produce error-containing proteins. Search for errors was vigorously pursued by measuring in great detail the kinetic properties (Km, Ki), molecular weight, electophoretic mobility, heat stability and antigenicity of various enzymes among different ages and species of animals. Most molecular properties of enzymes remain unchanged with ageing. In some cases, however, differences have been observed for heat sensitivity and antigenic response. In spite of some differences in the molecular properties of a few enzymes, there is enough evidence which negates the idea of errors in the proteins as function of age.

Gene regulation

This theory was proposed by Kanungo to explain the two important characteristics of the ageing process: a) the gradual decline in adaptability to the environment after attaining reproductive maturity; and b) the approximately fixed life-span for a species. According to this theory, senescence may result from changes in the expression of genes after reproductive maturity is reached. The basic assumptions of this theory are based on the sequential activation and repression of genes for various haemoglobin (oxygen-carrying proteins in RBC) chains during the gestational period in humans.

Each phase of life-span has a characteristic duration, rate and regulatory mechanism. The initiation, rate and duration of development and reproductive phases depend on unique sets of genes that are sequential-

ly activated and repressed. The products or by-products of the genes responsible for differentiation and growth, on reaching critical levels, stimulate certain unique genes responsible for the reproductive phase.

However, as a result of continued reproduction, certain factors may be depleted as they may not be replaced as fast as they disappear. Such factors may be of crucial importance for keeping certain genes expressed or repressed. They may also cause activation of undesirable genes and thereby lead to destabilization of expression of those unique genes which are required for reproduction, hence a gradual decline in reproductive rate with age.

One of the examples cited is the sudden death of the female octopus which lays eggs only once, broods them, reduces food intake and dies soon after the hatching of the young ones. Removal of the two optic glands after spawning prevents brooding, and the octopus continues to eat and to grow and increases longevity. It appears quite obvious that certain factors produced in the optic glands are responsible for brooding and cessation of feeding followed by senescence and death. Egg-laying may deplete certain factors which may in turn cause the optic gland to produce a hormone that causes behavioural change. A similar phenomenon is observed in salmon and certain insects. This theory also predicts that should the organism be able to replace the factors which become depleted due to continued reproduction, the reproductive period and perhaps life-span would be lengthened.

Coming to cellular theories, these relate to those changes which occur in structural and functional elements of cells with the passage of time. They also concern the biomolecules (proteins, carbohydrates, lipids, nucleic acids) after their synthesis is completed, suggesting that these changes impair the effectiveness of these molecules as a function of age.

Wear and tear

Accordig to the 'wear and tear' theory, living organisms are like machines—with repeated use, parts wear out, become defective and the machinery finally fails of function. This assumption is not entirely appropriate because organisms have a mechanism by which they can repair their damages, whereas machines do not. The basis for this theory originates from the observation that the life-span of poikilotherms (cold-blooded animals which can change their body temperature depending on the environmental temperature) is shortened by increasing the environmental temperature and prolonged by decreasing it. Indeed, the metabolic rate of chemical reactions is increased by increasing temperatures, and the reverse is true for low temperatures.

This phenomenon has also been observed in drosophila (fruitflies). The increased metabolic rate may shorten life-span by accelerating wear and tear. The life-spans of different animal species are inversely proportional to the basal metabolic rate (BMR). Basal oxygen consumption rates of short-lived animals such as rats and mice are much higher than those of long-lived animals such as elephants and humans. However, within the same species, it is difficult to correlate individual differences in life-span with the metabolic rate.

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Age pigments

Accumulation of lipofuscin (*lipo* from the Greek: fat; *fuscin* from the Latin: dusky) or age pigment is the most prominent age-associated change present in a variety of cell types from many organisms. It is predominantly deposited in non-dividing cells such as neurons (nerve cells) and cardiac myocytes (muscle cells) as a function of age. Lipofuscin is also deposited in dividing cells like liver, adrenal cortex and testis. Its accumulation is associated with the loss of cytoplasmic mass, mitochondrial number, rough endoplasmic reticulum and vacuolation of cytoplasm. The existence of a specific relationship between the rate of ageing and lipofuscin accumulation is demonstrated in the house fly (*Musca domestica*). For example, the rate of lipofuscin deposition has been inversely correlated with the life-span of many animals. The faster the rate of lipofuscin accumulation the shorter will be the life-span. The rate of lipofuscin deposition in the dog heart has been found to be approximately five times faster than in the human heart, a difference which roughly corresponds to that of the life span of these two animals. The origin of age pigments is not clear although a chemical hypothesis suggests that it is an end product of lipid peroxidation. The cause(s) for accumulation of age pigment remain(s) to be explored.

Free radical theory

The free radical theory of ageing postulates that free radical reactions (modified by genetic and environmental factors) are involved in ageing and age-related disorders. Free radical reactions are ubiquitous in living organisms. These reactions arise upon exposure to ionizing radiations, from non-enzymatic and enzymatic reactions, particularly those of the energy-gaining processes like reduction of oxygen to water during normal respiration. Free radicals are chemical compounds highly reactive due to the presence of extra electrons in the outer orbit. These radicals include O_2., HO., R. (any organic radical), RO., RO_2.. Although oxygen is essential for life, it also produces superoxides (O_2.) which are harmful due to their high random reactivity with other biomolecules. It will not be selfish to consider oxygen as a potentially dangerous friend.

The deleterious effects of free radical reactions can be repaired by the presence of antioxidants such as tocopherols (vitamin E), ascorbic acid (vitamin C), glutathione, glutathione peroxidase, superoxide dismutase,

elevated serum uric acid levels, carotenes (precursor for vitamin A). Antioxidants which are known to inhibit free radical reactions have been reported to prolong the life-span of various species such as roundworms, fruit flies and mice. There is evidence suggesting that the activity of superoxide dismutase (one of the antioxidant enzymes) is higher in long-lived animals such as humans than short-lived like rats and mice. This theory predicts that overprorudction of free radicals and/or reduction of their repair (removal) cause cell and molecular damage and ultimately ageing of the organisms.

Cross-linking theory

Many of the biological macromolecules develop cross-linkages or bonds between identical molecules or with different molecules with the passage of time. These linkages alter the physical and chemical properties of these molecules. Major support for this theory was provided by the work of Verzer on the extracellular fibrous protein collagen. It is synthesized in all cell types and is deposited extracellularly in all tissues. The structural unit of collagen is tropocollagen. It is a long (300 nm) and thin (1.5 nm in diameter) protein that consists of three coiled polypeptide subunits called a chain. Each ∝-chain contains 1050 amino acid residues. The three chains wind around each other in a right-handed triple helix which is held together by hydrogen bonds.

In collagen fibres, tropocollagen molecules pack together side by side and are stabilized by chemical cross-links between the chains. The mode of packing creates periodic striations in the structure of collagen fibres. The number of striations in the collagen of rat tail tendon and its thermal stability increase whereas solubility decreases with age. The increased cross-links in collagen would make it more insoluble and heat stable with ageing. Cross-linking agents are produced duirng normal metabolism. These groups react irreversibly with biomolecules like DNA, proteins, and lipids, and inactivate them, possibly reducing physiological competence with ageing. Cross-links are present not only in extracellular collagen but also in intracellular proteins as well as nucleic acids. The increase in cross-linking of chromosomal proteins with DNA makes the chromatin more condensed (tight). This is demonstrated by the decreased extractability of chromosomal proteins from chromatin with ageing.

The thymus (has been voted) as the 'clock' for immunological ageing

Among system levels theories, we first consider neuroendocrine control theory. The overall performance of an animal is closely related to the effectiveness of a variety of control mechanisms which regulate the interplay between different organs and tissues. The effectiveness of homeostatic adjustments declines with failure of adaptive mechanisms which may in turn lead to ageing and death. Adaptation to stress, either external (from environmental stimuli) or internal (from emotional, hormonal, immunologic and metabolic stimuli),depends on control mechanisms orchestrated by the nervous and endocrine systems. The activity of several endocrine glands (thyroid, adrenals, gonads) is controlled directly by the pituitary gland and indirectly by the signals this endocrine gland receives from nervous centres, primarily the hypothalamus. For efficient adaptation, nervous and endocrine signals must be synchronized and responsive to the needs of the many functions they regulate. However, with ageing, hypothalamo-pituitary signals are lost or changed which may result in decreased function and increased pathology of most organs and tissue systems.

Immunological theory

The immune system protects the individual from a variety of potentially harmful substances and organisms. Several organs such as bone marrow, thymus, lymph nodes and spleen are involved in the production of immunological responses against the invading foreign (non-self) substances called antigens. Most proteins and some polysaccharides and nucleic acids act as antigens. Among major types of cells vital to an efficient immune response are T-cell and B-cell lymphocytes. T-cell lymphocytes are matured in the thymus, and B-cell lymphocytes are stored in lymph nodes and spleen. T-cells are responsible for cell-mediated immune responses which protect the body from pathogenic microorganisms, and also for rejecting foreign tissue grafts. The cytotoxic (killer) T-cells destroy the antigen by direct attack on antigens. Subsets of T-cells such as T-helpers, T-amplifiers and T-suppressors also influence immunological cascade reactions by interacting with cytotoxic T-cells and B-lymphocytes. B-cells secrete antibodies (immunoglobulins) which bind to antigens and thereby help to destroy them. Competence of the immune system declines with ageing.

This decline has been attributed primarily to a reduced function of the thymus and T-cells. The thymus begins to involute at adolescence and continues to atrophy throughout the life-span. The active thymic epithelial cells produce a specific protein, called thymosin, which promotes T-cell maturation. With thymic involution, the thymosin level declines and this, in turn, diminishes the T-cells' ability to destroy foreign substances. For several reasons,it has been proposed that the thymus gland may be the 'clock' for immunological ageing.

At this moment, it is difficult to put all the data together and derive a unified conclusion. The basic cause may underlie at the genetic level in conjunction with the extrinsic and intrinsic factors. The concept of the role of a single factor pervades many areas of biologic study and gerontology is no exception. It is unlikely that a single 'triggering' event is responsible for the ageing of the organism but, rather, that ageing entails numerous and complex interactions at different levels. MMI