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Biology and Genetics of Ageing

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A) General:

The ageing process may derive from changes occurring in parallel in different tissues due to intrinsic cellular mechanisms or changes in one tissue may be predominant. Some authors argue that aging is located within one tissue such as the brain, while others defend that aging originates in all tissues. Results from the model system C. elegans indicate that a few lineages in mosaic organisms confer longevity perhaps due to endocrine signals. Neurons and the brain might play a regulatory role in aging.), at least to some degree. Some results from mice also suggest the existence of systemic factors in aging, but only to some degree. Although this debate has not been settled yet, it appears that intrinsic cellular mechanisms play a role in aging, though these can be modulated by extracellular factors like hormones. Aging has been defined as the collection of changes that render human beings progressively more likely to die (Medawar, 1952). Indeed, one hallmark of aging in humans and in many other species is an age-related increase in mortality rates shortly after maturity. Mathematically, aging can be quantified from mortality curves. Aging can also be defined as a progressive functional decline, or a gradual deterioration of physiological function with age, including a decrease in fecundity (Partridge and Mangel, 1999), or the intrinsic, inevitable, and irreversible age-related process of loss of viability and increase in vulnerability (Comfort, 1964). Clearly, human aging is associated with a wide range of physiological changes that not only make us more susceptible to death but limit our normal functions and render us more susceptible to a number of diseases. In humans, albeit some functions like hearing and flexibility begin to deteriorate early in life (Bowen and Atwood, 2004), most of our body's functional decline tends to begin after the sexual peak, roughly at age 19. Contrary to demographic measurements of aging that show mortality rates increasing exponentially, the human functional decline tends to be linear. Succinctly, aging is characterized by changes in appearance, such as a gradual reduction in height and weight loss due to loss of muscle and bone mass, a lower metabolic rate, longer reaction times, declines in certain memory functions, declines in sexual activity--and menopause in women--, a functional decline in audition, olfaction, and vision, declines in kidney, pulmonary, and immune functions, declines in exercise performance, and multiple endocrine changes. Although the immune system deteriorates with age, called immunosenescence, a major hallmark of aging is an increase in inflammation levels, reflected in higher levels of circulating proinflammatory cytokines and that may contribute to several age-related disorders such as Alzheimer's disease, atherosclerosis and arthritis (Franceschi et al., 2000; Bruunsgaard et al., 2001). In 1961, and in contradiction to what was thought at the time, Leonard Hayflick and Paul Moorhead discovered that human cells derived from embryonic tissues could only divide a finite number of times in culture (Hayflick and Moorhead, 1961). Because senescent cells can secrete proinflammatory cytokines and other factors that disrupt the tissue microenvironment, they may contribute to disruption of cell and tissue function. Even a

small percentage of senescent cells, in fact, may interfere with tissue homeostasis and function (Shay and Wright, 2000). Indeed, some evidence exist that senescent cells contribute to age-related pathologies such as osteoarthritis (Martin and Buckwalter, 2002; Price et al., 2002) and to skin aging (Giacomoni et al., 2000). Manipulations in C. elegans that extend longevity show a strong correlation with resistance to stress. Drosophila too some mutations can increase longevity and augment stress resistance. Finally, cells taken from patients with progeroid syndromes are more susceptible to stress.). In 1972, James Watson called this the end-replication problem (Watson, 1972). Olovnikov proposed the end-replication problem resulting in telomere shortening with each round of replication and that, because the DNA is replicated during cell division, this mechanism could be the cause of replicative senescence (RS) (Olovnikov, 1971 & 1973). Soon after, studies by Leonard Hayflick and colleagues found that the nucleus controls RS (Wright and Hayflick, 1975; Hayflick, 1994).

Aging, like many other biological processes, is subject to regulation by genes that control pathways that have been conserved during evolution to keep the balance between apoptosis and growth, cell division. There are some very important pathways, which have been found to be involved in aging and longevity. Among them the insulin/ IGF-1 pathway, the mTOR(mammalian target of rapamycin, a protein formation signal) pathway and p53 pathways are mostly conserved pathways that impact upon longevity and aging-related diseases such as cancer. pRb is a central regulator of cell cycle progression and its state of phosphorylation determines cell cycle regulation. Hyperphosphorylated pRb allows the cell cycle to proceed while hypophosphorylated pRb prevents cell cycle progression. Presumably, pRb operates through inactivation of the E2F family of transcription factors, responsible for transcription of several genes involved in G1/S transition and DNA synthesis. Cyclin-dependent kinase inhibitors (CDKIs) inhibit the activity of CDKs. One of such proteins is p16INK4a, that disrupts and inhibits the activities of CDK4 and CDK6, thus preventing cell cycle progression. Another important CDKI is p21WAF1, which also has the ability to block the cell cycle by inhibiting CDK2, CDK4, and CDK6 and thus preventing pRb phosphorylation. p21WAF1 can induce senescence independently of p16INK4a. P21WAF1 is induced by p53 to trigger RS. p53 is a transcription factor capable of acting both as a transcriptional activator and suppressor. Overexpression of p53 leads to cell cycle arrest or apoptosis. The induction of p53 by DNA-damaging agents led to the suggestion that p53 is a checkpoint factor that prevents cells from accumulating mutations by inducing apoptosis or growth arrest. p53 may help maintain genetic stability, Increased levels of p53 have been associated with critically short telomeres . Thus p53 is probably responsible for recognizing dysfunctional telomeres--e.g., critically short telomeres--as DNA damage and triggering RS. Indeed, activation of p53 occurs as cells approach senescence. We know that most of all cancers arise in the last quarter of life span with the frequency increasing exponentially with time. They show accumulated mutations in critical genes especially in the p53, gene, they show hypermethylation of genes critical to cell survival in individual cells over a lifetime.. Given the crucial role of the p53 in apoptosis, ageing tumour prevention, a decline in p53 activity at older ages and an increased hypermethylation in cells of animals could contribute to the observed increases in tumorigenesis and provides a profound understanding between tumour and aging. In addition, we see the accumulation of DNA mutations over lifetime, and the increased expression of risk genes or a decreased expression of genes, involved in detoxification. Demethylation of risk genes and hypermethylation of protective genes, accumulation of mutations in protective genes is a common event in ageing.

Telomere Theory of aging

One possible cause of aging is the shortening of the telomeres at the end , which loss of DNA bases . As the cell divides over and over, these telomeres become shorter and shorter. Telomeres are actually a loop-like structure which is associated with an assortment of proteins, the most notable of which are the Telomeric Repeat-binding Factors (TRFs). TRF1 regulates telomere length, assisting the telomerase enzyme. TRF2 models the telomere into the T−loop structure. (Loss of TRF2 from telomeres directly signals apoptosis). Telomerase is a reverse-transcriptase enzyme that elongates the telomeres and thus corrects the normal telomere erosion
Only in germ cells and in tumour cells one important enzyme can be expressed, the telomerase. This enzyme lengthens the telomeres by adding new processed DNA bases into the loop structure. Telomerase is a reverse-transcriptase enzyme that elongates the telomeres and thus corrects the normal telomere erosion.

Progeroid syndromes

Progeroid syndromes, as they are called, are rare genetic diseases that originate a phenotype that resembles accelerated aging. The three most studied such syndromes are Werner's (WS), Cockayne, and Hutchinson-Gilford's syndrome. Though patients with Down syndrome or trisomy 21 often also exhibit progeroid features this disease has not gathered as much attention from the perspective of aging research as the other three syndromes named above. In particular patients with WS exhibit striking features resembling accelerated aging and show an early onset--compared to normal aging--of multiple age-related diseases like diabetes, cataracts, osteoporosis, baldness, and atherosclerosis.

Werner's syndrome

WS is an inherited disease, in which patients are borne with normal telomere length, but they shorten faster then those of non Werner patients and lead to accelerated aging. WS is associated with early onset of very many age-related diseases and most closely represents accelerated aging of any of the segmental progerias. The majority of Werner's victims are Japanese (attributed to inbreeding). Werner's Syndrome is due to a defect or deletion of a single gene (WRN), resulting in defects of both telomeres and DNA repair. The WRN gene is a member of the helicase family that causes DNA to unwind, which is a requirement for most forms of DNA repair. Defective WRN protein results in a reduction of p53-mediated apopoptotic signaling.

Hutchinson-Gilford Progeria Syndrome (**HGPS**, "childhood progeria")
These children are born with short telomeres. Childhood progeria occurs once per 4−8 million births. The disease is caused by a deletion mutation in the gene for lamin A, a

filament protein in the nuclear matrix and nuclear lamina that is required for DNA replication and nuclear organization. The point mutation results in a prelamin A that cannot be converted to lamin A because it is missing 50 amino acids. At age 5 the telomeres of a Hutchinson-Gilford syndrome child are about as long as those of a very elderly person.

Genes

The greatest evidence in favor of seeing aging as having a unifying core is the way single genes can modulate the aging process. According to the GenAge database, hundreds of genes that when individually manipulated can alter aging and/or lifespan have been identified in model organisms. The plasticity of lifespans in invertebrates shows how one or a few genes can regulate the entire aging process. The plasticity of lifespans in invertebrates shows how one or a few genes can regulate the entire aging process. For example, in worms, single gene mutations can extend lifespan by almost 10-fold, in mice, there are several examples of single genes that can extend longevity, in some cases by around 50%, increase the MRDT, and delay the onset of multiple age-related changes and diseases.

A research group could show, that it is possible to slow aging in laboratory rats just by making them eat less calories while maintaining normal levels of proteins, vitamins, and minerals (McCay et al., 1935). This process became known as caloric restriction (CR) and appears to work in many animals; it has been particularly well-studied in mice. From mice, we know that CR not only increases longevity by up to 50% but it also postpones or diminishes the incidence of most age-related diseases, decreases the rate of aging.

Oxidative Stress, IGF-1 –receptor and longevity

Oxidative stress seems to reduce the lifespan. Latest after Denham Harman's publication of the "Free Radical Theory of Aging" of in the early Fifties of the last century we know, that free radicals, produced in the cell mitochondria or introduced into the cells by physical alteration of the single oxygen molecule, the hydrogen molecule, the nitro molecule and others damage cells processes, which are conserved for life maintenance. P66Shc regulates both life span (in mice) and sensitivity of cells (human or mouse cells in culture) to oxidant stress. Mice that lack p66Shc live almost one third longer than do control animals. Certain mutations of the protein p53 lead to accelerated aging, whereas deletion of the gene for the protein p66Shc results in increased longevity. A possible function of p66Shc is to transduce signals from p53 that accelerate the rate of aging by inhibiting the expression of genes for antioxidant proteins. Removal of the p66*Shc* gene, which encodes an adaptor protein for cell signaling, extends lifespan by 30% in mice and confers resistance to oxidative stress. The absence of p66Shc correlates with reduced levels of apoptosis.

In Caenorhabditis elegans, the downregulation of insulin-like signalling induces lifespan extension (Age) and the constitutive formation of dauer larvae (Daf-c). This also causes resistance to oxidative stress (Oxr) and other stress stimuli and enhances the expression of many stress-defense-related enzymes such as Mn superoxide dismutase (SOD) that functions to remove reactive oxygen species in mitochondria. The daf 2 gene mutation in C. elegans, a gene, which is comparable with the human IGF-1 Receptor gene and

which is involved in antioxidative action, shows a 30 % increased lifespan in that species.
In human we found the following: Sequence analysis of the IGF1 and IGF1 receptor (IGF1R) genes of female centenarians showed overrepresentation of heterozygous mutations in the IGF1R gene among centenarians relative to controls that are associated with high serum IGFI levels and reduced activity of the IGFIR as measured in transformed lymphocytes. Thus, genetic alterations in the human IGF1R that result in altered IGF signalling pathway confer an increase in susceptibility to human longevity, suggesting a role of this pathway in modulation of human lifespan.

Aging is controlled by different mechanisms, which influence the speed of the aging process and which is determined by interindividual genetic variants. When we age, we register an average increase of LDL cholesterol and enhanced low density lipoprotein (LDL) oxidation and atherosclerosis. Therefore subjects over 80 years without cardiovascular disease provide a model to investigate the protective factors against atherosclerosis.
One important antioxidative enzyme is transported with the HDL protein and is called paraoxonase (PON1), a high density lipoprotein (HDL)-bound enzyme, prevents LDL oxidation. There is a strong evidence that in healthy Sicilians ageing may be characterized by a low frequency of PON1 (-107)T 'risk' allele and by an high frequency of favourable genotypes such as (-107)CC, influencing PON1 activity and HDL-C levels. The non enzymatically formation of sugar bounds leads to increased molecular instability, forming cross- linbking of the DNA and posttranslational transcripts. Disorders in the insulin and lipid metabolism, caused by genetic variants of the genes, involved in this part of the metabolism cause higher glycolisation and tissue damage. The methylated CpG dinucelotids of DNA regulate the genetic activity , coupled with deacytilation or acetylation of the chromation , suggesting that the switch on and off of a particular gene is determined by this methylated or demethylated dinucleotids, while the NAD dependent acetylation or deacetylation regulates the chromatin packing or depacking (silencing of the genome), including SIR proteins as regulator. Furthermore methylation is involved in cancer formation and neurotransmitter and steroid hormone elimination, as wll homocysteine formation, suggesting a most important role of methylating and de-methylating enzymes in the aging process. Genetic variants of the methylating enzymes lead to individual expression pattern of this enzymes. The involvement of histone modification in the regulation of gene transcription has been widely demonstrated. Histone modification, specifically histone acetylation, is important in the regulation of the mammalian circadian clock. The key proteins that regulate the circadian clock (Clock and Bmal1) drive the transcription of three period genes (Per1, 2 and 3) and two cryptochrome genes (Cry1 and Cry2). The transcriptional silence of tumour suppressor genes by promoter CpG islands Hypermethylation can contribute to oncogenesis. Many recently discovered genes, known by such cryptic names as *daf-2, pit-1, amp-1, clk-1* and *p66Shc*, have been found to affect stress resistance and life span in laboratory organisms, suggesting that they could be part of a fundamental mechanism for surviving adversity. Sirtuin genes function as anti-aging genes in yeast, Caenorhabditis elegans, and Drosophila. To understand their cellular function in these age-related diseases, identification of sirtuin targets and their subcellular localization is paramount. SIRT3 (sirtuin 3), a human homologue of Sir2 (silent information regulator 2), has been genetically linked to lifespan in the elderly. However, the function and localization of this enzyme has been keenly debated. A number of reports have indicated that SIRT3, upon proteolytic cleavage in the mitochondria, is an active protein

deacetylase against a number of mitochondrial targets. The NAD requirement for sirtuin function indicates a link between aging and metabolism, and a boost in sirtuin activity may in part explain how calorie restriction extends life span. In mammals, one of the substrates of the SIR2 ortholog, SIRT1, is a regulator of mitochondrial biogenesis, PGC-1alpha. Indeed, the putative SIRT1 activator resveratrol has been shown to stimulate mitochondrial biogenesis and deliver health benefits in treated mice. Sirtuins are NAD+-dependent enzymes that have been implicated in a wide range of cellular processes, including pathways that affect diabetes, cancer, lifespan and Parkinson's disease.

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