How Does Ageing Occur?

Comparing Biological Theories of Ageing

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How does ageing occur? It seems like it a simple question but, in truth, it is anything but. The science behind ageing is far more complex than you might expect and, therefore, it is still very much a matter of contention. However before we can discuss how ageing occurs we must first define ageing. The definition of ageing used by gerontologists, scientists in the field of ageing, is "an age-dependent or age-progressive decline in intrinsic physiological function, leading to an increase in age-specific mortality rate" [1] and this definition will form the basis of my essay. There are many differing theories of how the process of ageing occurs. However, it is mostly thought that ageing is not one simple phenomenon that is caused by one single event, such as a single gene or the deterioration of one system, but is actually a complex process that is made up of many factors that together contribute to ageing. This is thought to be the case because it is unlikely that a single event would cause many tissues, organs and systems to deteriorate and, therefore, it is more likely that ageing is multifactorial because each factor can cause the deterioration of a different system or organ. Therefore, it is likely that ageing is due to a combination of the various theories and, therefore, I think it would be wrong to view these theories as being totally separate from each other.

One of the most popular and well known theories of cell ageing is the Telomere Theory of Ageing. In 1965 it was discovered [2] that human cells, in culture, cease to divide after a set number of cell divisions. When this happens it is known as cell senescence or the Hayflick limit. In eukaryotic cells (cells that contain a nucleus) there are repetitive DNA sequences found at both ends of chromosomes called Telomeres [Figure 1]. In humans, telomeres are the sequence of bases TTAGGG repeated for up to fifteen thousand bases [2]. Telomeres are present in order to maintain chromosomal stability and so it is theorised that without them chromosomes would fuse during mitosis (cell division). With each DNA replication approximately 25 to 200 bases [3] from the non-coding DNA of the telomeres are lost because the replicative DNA polymerases that replicate DNA cannot start from the very end of each DNA strand. It is possible that this shortening of the telomeres works as a potential 'countdown' to cellular senescence where after the telomeres run out the cell stops dividing to prevent loss of the important genetic material. The theory proposes that the accumulation of senescent cells within the body leads to the decline in function that we call ageing. Evidence to support this claim is that genetically modified mice with lengthened or shortened telomeres have longer or shorter lifespans respectively and that faulty telomeres increases the ageing rate.[4] Telomeres are made by the telomerase enzyme and it is thought to be the key to immortal cells. Normal, mortal cells do not contain telomerase so their telomeres are shortened with each DNA replication while immortal cells, such as stem cells, germ cells, and T lymphocytes, all contain telomerase which either slows down the shortening of telomeres or keeps the telomeres at a constant length and

Figure 1

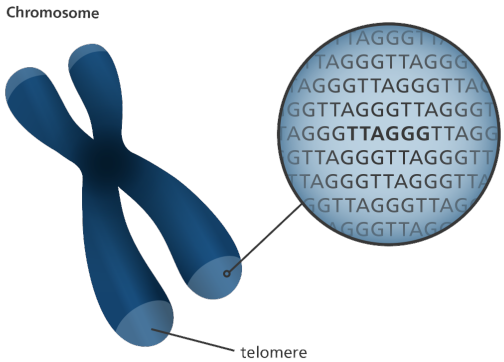


Diagram of telomeres on the ends of a chromosome

What is a telomere?; yourgenome.org < http://www.yourgenome.org/facts/what-is-a-telomere> [accessed 8th September 2016]

therefore these cells never become senescent. Cancer cells also express telomerase which could explain how they can divide indefinitely. [2] Further evidence for this theory is that mice who lacked telomerase aged prematurely but if the enzyme was replaced the ageing was reversed and they "bounced back to health" [5]. This possibly may lead to an anti-ageing treatment for humans by "reawakening" the telomerase in human cells however it is thought that this may lead to more tumours because cancer cells express telomerase which could explain how they can divide indefinitely. [5] I think this theory holds great promise because of the evidence we do have, such as telomeres of constant length in immortal cells and premature ageing of mice without telomerase, however I think further experimentation is required to say it is a definite cause of ageing.

The Free-Radical theory of ageing is the one theory that appears to be quite widely accepted as a possible cause of ageing. [2] This theory was developed in 1956 by Dr. Harman and it states that accumulation of oxygen free radicals, in particular Superoxides [Figure 2], within cells is the cause (or a cause) of the decline in function that is associated with ageing. Free radicals are molecules with an unpaired electron in the outer shell. These free radicals attack molecules in the cells such as nucleic acids, sugars, lipids, and proteins. This can cause damage to DNA, cell membranes, enzymes and structural proteins. This causes the cells, and eventually the organs made up of those cells, to not function correctly which causes the deterioration of function that comes with ageing. The theory was then revisited by Harman in 1972 when it was proposed that mitochondria make these oxygen free radicals (Superoxides) as a byproduct of normal function. The way this works is that the electrons can "leak" from the electron transport chain in mitochondria that is required for respiration and these electrons reduce the stable oxygen molecule to the superoxide free radical. [6] Organisms contain enzymes that act as antioxidants which counteract the negative effects of the free radicals and this allowed experiments to be held. In these experiments, rats were fed extra antioxidants and these rats had longer lives on average than the rats who weren't fed antioxidants. [7] This could show that slowing the accumulation of free radicals with antioxidants increases average life expectancy and therefore free radical accumulation is a cause of ageing. [7] However it is entirely possible that this is correlation and not causation and the increased life span was due to some other unknown, beneficial effect of antioxidants and not the reduced number of free radicals.

Figure 2

Lewis structure of a Superoxide ion.

Wikipedia; Superoxide < https://en.wikipedia.org/wiki/Superoxide> [accessed 8th September 2016]

Another theory known as the Mitochondrial Theory of Ageing expands on the Free Radical Theory by stating that it is the accumulation of free radicals damaging the mitochondria that is a cause for ageing. The theory states that when mitochondrial DNA is damaged by free radicals the mitochondria ends up producing more free radicals as a result of the damage. This is because the free radicals damage the mitochondrial DNA which introduces "altered enzyme components into the electron transport chain" [2]. This means more electrons can leak out of the electron transport

chain and, therefore, make more superoxide free radicals. These new free radicals damage the cell more and this starts a “vicious cycle” of damage leading to more free radicals leading to more damage.[8]

Ageing and then, ultimately, death has also been proposed to be caused by the natural decline in body’s immune system in the Immunological Theory. The immune system is the body’s main method of defence from pathogens. The theory states that the immune system is programmed to decline after puberty. The cause of this decline is thought to be thymic involution where the thymus, an organ in the lymphatic system that is responsible for the production of T-lymphocytes, shrinks after puberty. [9] The thymus “reduces from two hundred to two hundred and fifty grams at birth and then shrinks to around three grams by age sixty”. [8] This shrinking of the thymus means fewer T-lymphocytes, a type of white blood cell that is required for immune response, mature and therefore as you age the immune system weakens. The weakening of the immune system with age means the elderly are more susceptible to infectious diseases. One issue with this theory is that there is no agreed on cause of the decline in the immune system although, to me, thymic involution seems like the logical cause. I personally think that this theory is a likely cause of ageing however I think it is very likely to be just one of the mechanisms within the body that contributes to ageing.

Not to be confused with the Immunological theory is the Autoimmune theory which proposes that the immune system’s ability to distinguish between self and foreign antigens declines with age and so it becomes self-destructive and begins to attack and breakdown the organisms own cells. [10]

The DNA Damage Theory states that ageing is caused by an accumulation of DNA damage and mutations in DNA. [11] DNA damage is when the double helix structure of the DNA changes, such pyrimidine dimers, where two pyrimidine bases pair rather than the one purine and one pyrimidine, apurinic sites, where a location in DNA doesn’t have a purine or pyrimidine base, and chemical adducts, where two or more molecules in DNA form an addition product [11]. Mutations are when the sequence of bases changes such as point mutations where one base is replaced by another, base addition where one base is added and base substitution where one base is removed. In the cells of organisms DNA damage and mutations can accumulate with age. Some of this damage is repaired naturally in the cell but some of it is not. The accumulation of these mutations and damages cause cell function to decline and it is this reduction in cell function that causes the decline in physiological function that we associate with ageing. [7] Evidence of this theory is that “deficiency in DNA repair mechanisms caused accelerated ageing in mice” and many premature ageing diseases in humans, including Werner Syndrome and Bloom Syndrome. As a consequence this premature ageing must be caused by accumulation of mutations and damage in DNA. [4]

In particular, ageing is theorised to be caused by reduction of function in mitochondria due to the accumulation of damage in mitochondrial DNA (mtDNA). Mitochondrial DNA repair mechanisms are less efficient than repair mechanisms for nuclear DNA and mtDNA does not contain the

protective histones that are found in nuclear DNA and therefore it is more vulnerable to DNA damage. [4] If mitochondria do not function correctly then other cells won't get the necessary energy to function so other organs and tissues will experience a decline in function so the organism ages and then dies.

The Neuroendocrine Theory states that the natural decline in the endocrine system and nervous system is a cause of ageing. With age the nervous system and endocrine system deteriorates as does the link between the two called the hypothalamo-pituitary-adrenal (HPA) axis. [2] This results in "a loss of homeostasis" (maintaining a stable, constant internal environment for the body) and a "higher risk of death". This deterioration causes changes in the regular functions that are "crucial for 1) coordination and responsiveness of different systems to the external environment; 2) programming physiological responses to environmental stimuli; and 3) the maintenance of an optimal functional status for reproduction and survival" [2]. The decline in the endocrine and nervous systems changes how efficient the body is at surviving by adapting to stress among other things such as "reproduction, growth, and development". This theory proposes that the fall in the body's ability to survive by adapting to stress is what causes the decline in physiological function from our ageing definition. Eventually the body's ability to survive by adapting to stress will decline so much that the organisms dies. Some evidence that there is a decline in the endocrine system is that the levels of hormones like testosterone, oestrogen and human growth hormone decrease with age.

Some gerontologists believe that the Neuroendocrine theory and the Immunological theory are linked so refer to the Neuroendocrine-immuno theory of ageing. This states that it is the decline in the nervous, endocrine and immune systems that, together, causes a great fall in the body's ability to survive by adapting to stress. This decline in the body's ability to survive by adapting to stress is caused by reduced defence from pathogens due to a weakened immune system and reduced ability to respond to stimuli due to the deterioration of the neuroendocrine systems. This fall in survivability is what causes ageing.

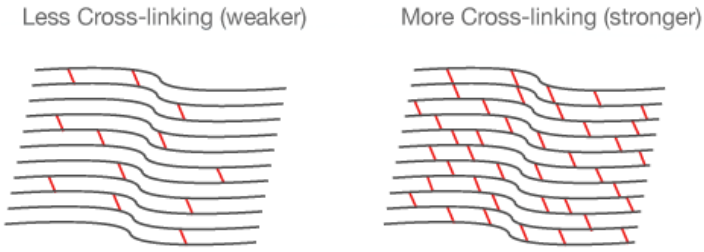
Another theory is the Gene Regulation Theory of ageing which proposes that cellular senescence is caused by changes in gene expression. [7] There is evidence to show that gene expression changes with age but I agree with the authors of 'The aging process and potential interventions to extend life expectancy' who state that it is unlike that there are "genes that promote senescence directly" [2]. I cannot find a huge amount of evidence to suggest this theory is correct and so gerontologist mostly think that ageing is a complex process influenced by many factors rather than a programmed process entirely controlled by genes

Another theory of ageing is the 'Wear and Tear' theory and it is relatively self-explanatory. It is a very general theory introduced in 1882 by Dr. August Weismann and it dictates that cells and tissues have important components that are damaged by overuse and by external factors. [8] This 'wear and tear' can be caused by toxins, radiation and ultraviolet light that we encounter naturally through life.

The loss of these important components causes the cells to become damaged and, therefore, not function correctly. It is the accumulation of damaged cells that causes the decline in function that is associated with ageing.

The Cross-Linking Theory proposes that cross-linking of proteins is the key to ageing. Cross-linking is when proteins form bonds with other molecules in the body or other proteins and, therefore, cannot function as normal. [7] These cross-links are usually made up by small sugars like glucose or fructose. Cross-linking can cause enzymes to denature because they disrupt the tertiary structure of the enzyme. This means the active site of the enzyme is no longer commentary to the substrate and so it is unable to bind with the substrate to form an enzyme-substrate complex and it is, therefore, unable to perform its function as a biological catalyst within the cell. As these cross-linked, non-functional proteins accumulate the cells and tissues become damaged and faulty. These damaged cells accumulate and this leads to the decline in function and increase in mortality rate that we associated with ageing. One piece of evidence that accumulation of cross-linked proteins causes ageing related problems is cross-links that build up with age in collagen, a protein present in skin. Collagen is a fibrous protein that is made up of collagen fibrils. These fibrils can move relatively freely giving skin elasticity but when cross-links form [Figure 3] between the polypeptides in the fibrils they cannot move as freely and so skin looses elasticity. This is thought to be the cause of wrinkles. It is also proposed that cross-links are a cause or the cause of cataracts, a condition of the eye that inhibits vision that is common in the elderly. [12]

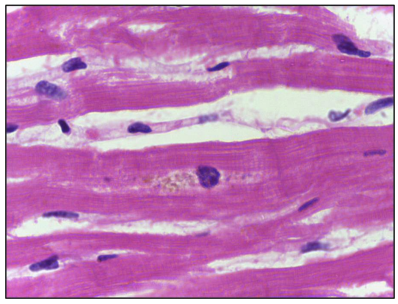
Figure 3



Diagrams to show how cross-links accumulate between the fibrils of collagen

Collagen Cross-Linking; laservue.com < https://www.laservue.com/keratoconus/corneal-collagen-cross-linking/> [accessed 8th September 2016]

Figure 4



Micrograph containing Lipofuscin granules stained purple.

Wikipedia; Lipofuscin <https://en.wikipedia.org/wiki/Lipofuscin> [accessed 8th September 2016]

The Waste Accumulation Theory proposes that one factor that contributes to ageing is the accumulation of waste from natural cell function. During a cell's lifetime it will create waste and not all of that waste can be eliminated from the cell. This waste can include certain toxins that, when they reach a certain concentration, can interfere with regular cell function and eventually kill the cell. [8] Lipofuscin [Figure 4] is considered to be evidence of this theory because it is a waste product that accumulates and leads to "granular yellow-brown pigment granules" that increase in size with age. [13] This evidence shows that waste products can accumulate with age and cause damage to the cell.

The final theory I will be discussing is the Death Hormone Theory. This theory, proposed by Dr Denckle, proposes that with age the pituitary gland, a structure in the brain that releases hormones, release decreasing oxygen consumption hormone (DECO). He proposes that this 'death hormone' prevents the body from using thyroxine. Thyroxine decreases metabolism and so when DECO prevents the body from using it the body's rate of metabolism changes and this increases the rate of ageing. [14]

Despite not being a theory of ageing, I feel that some of this essay must be dedicated to Caloric Restriction as it is a major discovery in the field of ageing. It has been found that reducing calorie intake while maintaining the necessary amount of nutrients increased lifespan. [2] Experimental data shows that a reduction in calorie intake of about forty percent increased maximum lifespan by about thirty to forty percent. [2] It is theorised that this occurs because neuroendocrine, immune and metabolic responses are all improved by caloric restriction. The improvement in the neuroendocrine and immune response means the body better protected from stress. Caloric restriction is also thought to reduce the amount of cross-linking that occurs [2] and slow the rate of oxidative damage from free radicals. [10]

There are many similarities and differences between the theories I have described above. One big difference has led to the theories being split into two main groups, the Programmed Theories and the Damage or Error Theories. [7] Programmed Theories propose that the ageing process follows a "biological clock" that acts as a countdown to ageing. Theories in this group include the Gene Regulation Theory because it proposes that ageing is caused by a programmed change in gene expression, the Telomere Theory because it states that telomeres work as a biological countdown to cell senescence and, as a result, ageing, the Immunological Theory because it states that ageing is due to the programmed decline in the immune system and the Neuroendocrine theory because it proposes that ageing is due to a fall in survivability after a programmed decline in function of the nervous and endocrine systems.[1]

Damage Theories or Error Theories propose that the ageing process is due to accumulation of damage from internal and external stressors. Theories within this group include the Wear and Tear theory, Free Radical theory, Mitochondrial theory, DNA Damage theory, Cross-Linking theory and the Waste Accumulation theory because they all propose that ageing is due to the cells being damaged by assaults from various stressors such as free radicals, cross-linked proteins, DNA damages and other external stressors. As I have mentioned before it is possible (and maybe even probable) that ageing is controlled by a mix of both Programmed and Damage theories since ageing is such a complex process.

Alternatively the theories can be grouped, due to their similarities and differences, into 3 separate groups. The first group is the organ theories which contains the immune, neuroendocrine and

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1 As a result of both the Neuroendocrine and the Immunological theories being considered Programmed Theories, the Neuroendocrine-Immuno theory is also classified as a Programmed theory.

neuroendocrine-immuno theories. These theories are grouped together because they are similar in that they all propose that ageing is due to the decline of a certain system. The second group is the physiological theories which contains the wear and tear, free radical, mitochondrial, cross-linking and waste-product accumulation theories because they all propose that cells are damaged by various stressors (free-radicals, cross-linking, waste products) and an accumulation of damaged or faulty cells is what causes the decline in function that we call ageing. The final group is the genome-based theories which includes the gene-regulation and DNA damage theories which both propose that ageing is due to changes in the expression of DNA either through a programmed change or an accumulation of damage and mutations.

One issue I have with this method of grouping is the difficulty in finding a group for the telomere theory because telomeres are part of the genome and thus it could be a genome-based theory however the telomere theory also relies on a accumulation of senescent cells so it could be a physiological theory. Despite this issue I prefer this method of grouping because it classifies the theories by what they suggest is the cause of ageing rather than whether they are simply programmed or due to damage.

One big area of overlap is between the Free Radical and Wear and Tear theories and the DNA damage theory. In the DNA damage theory it is proposed that DNA damage is caused by both exogenous damage, damage originating from outside the organism, and endogenous damage, damage originating from within the organism. Exogenous damage includes toxins, radiation and ultraviolet light which are the main causes of damage in the Wear and Tear theory and exogenous damage includes damage from free radicals (amongst other things) which is the basis of the free radical theory.

The scientific world of ageing is still very much an area of science which has a long way to go. A lot of these theories have promise but I don't think any of them have the definitive evidence to entirely prove that they are a cause of ageing. I'm very much in agreement with the authors of 'Cellular Aging: Theories and Technological Influence' when they say that after looking at the theories "none of them explains the process in its entirety" [8]. Obviously I can't say which theories are most likely to be correct without evidence but I do think that some theories describe the whole body nature of ageing better than others. Personally I think that theories that propose that ageing is a result of an accumulation of senescent, faulty or non-functional cells, such as the free radical theory, cross-linking theory, telomere theory, wear and tear theory, mitochondrial theory and the DNA damage theory, describe the whole-body sense of ageing better because faulty cells can accumulate anywhere in the body so they can cause many organs or systems to deteriorate while theories like the neuroendocrine theory or the immune theory only describe the breakdown of individual systems. However, that doesn't imply that the former set of theories are better in someway than the latter because it is entirely possible the neuroendocrine and immune theories cause the age dependant decline in function of the nervous, endocrine and immune systems while other theories, like the free-radical or DNA damage theories, cause the decline in function of other

parts of the body since ageing is such a multifactorial process. Further experiments must be done to test the theories and shed some light on this very complex process. The reason we must shed light like on the causes of ageing is not just to further human knowledge of our own bodies but to potentially increase lifespan, prevent diseases common in the elderly and help the fight against cancer.

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