

2019

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The Role of mtDNA Mutation and Mitochondrial Dysfunction on the Aging Process

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Table Of Contents

1. Abstract	2
2. Introduction	4

	1
a. Significance	4
b. Modern Theories on the Nature of Aging	6
c. How do Mutations Occur?	12
d. Relevance of mtDNA Degradation of the Aging Process	13
3. Methods	15
4. Results	16
a. Mitochondrial Function is Naturally Impaired in Aged Individuals	16
b. Artificial Impairment of Mitochondrial Function Artificially Accelerates Aging in Affected Organisms	18
c. Reversing or Inhibiting mtDNA Damage Can Delay the Onset of Old Age	21
5. Conclusion	24
6. References	25

Abstract

Statement of Research Problem, Question, or Hypothesis

Recent anti-aging research has investigated several functions suspected of being responsible for the development of old age, with one being the impact of mutations in the mitochondrial DNA (mtDNA) and subsequent mitochondrial dysfunction on the onset of aging.

The objective of this review is to analyze whether or not there is enough evidence to state that mtDNA mutation is responsible for aging, and if so, if there exist methods capable of preventing such damage.

Context of the Research Problem, Question, or Hypothesis

Aging, characterized by a gradual decline in physical capability along with a similarly impaired ability to withstand external stressors is the primary cause of human death in the modern day, increasing susceptibility to age-related diseases including cancer, heart disease, diabetes, and neurodegenerative diseases. Though new advancements in medical technology are being achieved daily, the technology necessary to significantly delay aging in human beings does not yet exist, though modern-day advances makes the likelihood of eradicating aging in the near future fairly optimistic. Though new, more effective methods are still emerging, procedures that restore mitochondrial function have the potential to contribute against age-related diseases, and even extend the maximum human lifespan indefinitely

Methods

In order to address this problem, this review gathers information through a systemic analysis of existing experiments and observational studies already published in scientific journals. Papers that were considered acceptable for this study were limited to published scientific articles that either analysed the macroscopic effects of mitochondrial dysfunction, applied treatments designed to prevent or reverse mtDNA damage, or illustrated relevant background information in further detail.

Results

The data collected from analyzed experiments seem to support the idea that mtDNA damage is a contributor to the onset of old age. Not only is mitochondrial dysfunction much

more present in aged organisms than it is in youthful ones, but experiments that artificially impaired mitochondrial function in rats resulted in organisms that experienced an accelerated rate of aging. Furthermore, organisms subjected to methods that improved mitochondrial stability were shown to be much healthier overall, with a maximum lifespan somewhat greater than that of the wild-type.

Conclusion

The results observed in this review are quite similar to the results of earlier reviews on similar topics. If this research was to continue, one major experiment would be to observe if there were any physical or mental changes in mtDNA fortified organisms, or if treated organisms were more likely to develop any outstanding health problems. Though no experiments listed in the review noted any deleterious side-effects on mtDNA-fortified animals, future experiments may find something if they were specifically looking for it. In addition, future experiments could go more in depth, and take a much more detailed look at any anti-aging procedure.

Significance

Currently, aging and the various physiological complications that arise as a consequence of aging is the single largest contributing factor to human mortality. Worldwide, aging and associated negative physical side-effects are responsible for roughly twice as many deaths as all other causes of mortality combined, ending almost 100 thousand lives each day. However, the average proportion is even greater in developed “First World” countries, with approximately 90 percent of all deaths attributed to complications surrounding old age. According to the Center for Disease Control, out of the ten leading causes of death throughout 2016, only three (accidental injury, nephrotic syndrome, and suicide) existed independently of any significant age-related

factor. (“Leading Causes of Death” 2017) The other seven conditions (heart disease, malignant neoplasm, lower respiratory disease, cerebrovascular disease, Alzheimer’s, diabetes, and influenza) can be considered age-related diseases, or diseases that occur with significantly increased frequency in aged organisms (Wilson et. al 2008). Furthermore, due to advances made in modern medical technology and subsequent increases in global longevity, the amount of seniors within the global population is expected to significantly increase within the next few decades. While the amount of individuals aged 65 or over currently represents approximately roughly 8.5 % of the world’s population, current projections estimate that the older population will increase by roughly 1 billion people by the year 2050, making up just under 17% of the global population (He et. al 2016). Unfortunately, the relationship between the age of an individual and the likelihood of the development of age-related diseases suggests that a far greater proportion of individuals will suffer from such diseases in the near future. If projections of the global population are accurate, and if human beings continue to experience age at the same rate, more time and resources are going to have to be developed by the healthcare field in order to properly treat a growing number of patients that have fallen victim to age-related diseases. Therefore, in order to address these issues, scientists are beginning to conduct research designed to control and inhibit the process of aging.

Though many experts are currently optimistic of humanity’s ability to eventually inhibit or even negate the negative physical consequences of aging, modern science is currently unable to do so at this time. Though research into anti-aging technologies is currently in a nascent state, what progress has been done so far seems to suggest that human ageing is predominantly caused by a gradual accumulation mutations and errors in an organism’s genetic structure, including mutations and errors sustained by DNA in the mitochondria (Szczepanowska and Trifunovic

2017). Unfortunately, a definite consensus on the specific causes of aging have yet to be decided, as the scientific community currently maintains several conflicting theories, though each theory has some level of controversy around it, with no research on any theory being able to prove definite and conclusive proof. Therefore, in this paper, some of the more popular theories used to explain the ageing process will be explored, though most of the research that will be examined in this paper will be on the overall effect of gradual mitochondrial DNA (mtDNA) damage on the physical health of an organism. In addition, this paper will attempt to provide a review of existing studies that have both explored ways in which cellular dysfunction as a result of mtDNA damage could be treated or reversed, or have additional insight into the theory that the aging process is significantly influenced by damage to mtDNA.

The primary goal of this paper is to both examine the effectiveness of current methods designed to ameliorate the effects of mitochondrial dysfunction through inhibition of mtDNA damage, and to answer whether or not such dysfunction could be considered a significant contributor towards aging. If mitochondrial damage and dysfunction is responsible for the aging process in humans, and techniques exist by which one can control or inhibit the development or the expression of this damage, then any treatment that protects the mtDNA should result in a substantial increase in both average and maximum human lifespan. Furthermore, due to the fact that mitochondrial dysfunction is linked to Alzheimer's (Lunnon et. al 2107), sarcopenia (Pestronk et. al 2017), and other age-related diseases, any methods that have the ability to maintain mitochondrial function should be able to have a beneficial effect on such diseases. The ultimate goal of this paper is to potentially assist in the identification of one or more processes that can prove capable of either postponing or outright preventing the progression of the natural ageing process in order to both extend and improve the overall quality of human life.

For the purposes of this review, “aging” does not simply mean the process of getting older. Instead, the term is defined as the gradual development of general mental and physical changes that are experienced by an organism as a result of a set of deleterious physiological effects experienced within a cell. This review will mainly focus on physical aspects of aging, and will not discuss its social or emotional aspects.

Modern Theories on the Nature of Aging

Though a great deal of research has been conducted in order to understand and explain the process of aging, modern science is currently unable to agree on a single unified theory as many of these theories are either unable to explain certain aspects of ageing to a fully satisfying degree, or are outright contradicted by further experimental study. Challenges faced by these theories include outlining a single unique biochemical process responsible for aging within an organism, defining the exact purpose of the aging process, and stating whether or not there is an absolute limit to how long any given organism can live.

Modern theories that attempt to explain the aging process generally fall under one of two major categories, one of which is the Programmed Theory of Aging, a category that encompasses all theories that subscribe to the idea that aging is a “pre-programmed” biological pathway, comparable to similar pathways that influence childhood growth and development (Jin 2010). Explanations that fall under the Programmed Theory of Aging are based on the idea that aging is caused by internal cellular and genetic changes that directly and purposefully inhibit systems responsible for cellular maintenance and repair. One such theory is the Endocrine Theory of Aging, in which the aging process is regulated and controlled by hormones, with the insulin/IGF-1 signaling (IIS) pathway playing a key role in aging regulation. Studies of the effect of the IIS pathway in model organisms seem to support this theory, as mutated organisms with a down-

regulated IIS pathway demonstrate much longer lifespans (Heemst 2010). This increase in lifespan is believed to be due to IIS inhibition resulting in the activation of “master switches” known as Class O forkhead box transcription factors (FoxOs) capable of suppressing inflammation and enhancing cellular resistance (Horst, 2007). However, it is still uncertain whether FoxO transcription factors are responsible for aging themselves, or whether their activation merely inhibits a secondary pathway that is. Finally, the Immunological Theory states that the immune system is programmed to deteriorate just after puberty, leaving the body vulnerable to attack by opportunistic disease and promoting cellular stress, leading to aging and death. While it is well-known that immune response does decline after puberty, this age-related impairment of the effectiveness of the immune system could be a symptom of old age, and not necessarily its cause.

Proponents of programmed aging believe that aging is an intentional process designed to ensure the eventual death of an individual organism, ensuring that the individuals constituting any population are always replaced, ensuring that there are always resources available for new, more finely adapted organisms. Furthermore, proponents use the relatively uniform nature of maximum lifespan of organisms within a species to further support the idea that the aging process is predetermined, as there would be a great deal of variability in the rate at which individuals within a species age if aging was determined by an accumulation of genetic damage (Prinzinger 2005). However, the Programmed Theory of Aging has had several issues raised against it that work against its consideration as a complete theory (Kowald and Kirkwood 2016). One of the first arguments against programmed aging is the fact that the vast majority of organisms do not survive in the wild for long enough to ever experience old age, succumbing to disease, injury, or predation long before it reaches the end of its biological lifespan (Berry and

Bronson 1992). The virtual absence of senescent organisms in the wild contradicts the idea of aging being an intrinsic part of the genome. It is very unlikely that a pre-programmed biochemical process that was unlikely to ever be expressed in the vast majority of all organisms would remain for very long within a species' genetic code, and even more unlikely that such a process would be present in nearly every single organism on the planet (Berry and Bronson 1992). Furthermore, if aging was actually a pre-programmed biological process, it should be possible to identify one or more genes within the aging pathway and experimentally inhibit their action, developing organisms with the potential to live indefinitely. Unfortunately, though several genes have been located with the potential to influence aging in some way, scientists have been unable to identify one or more genes capable of preventing the aging process entirely.

The idea that aging is an intentional process designed to facilitate the constant presence of new, more finely adapted organisms within a population by cycling out individual organisms after a set period of time would be valid only if evolution worked towards the benefit of a population over the benefit of any one individual organism. If evolution favored the individual over the group, any individual organism with the ability to live and produce offspring indefinitely would have a definite advantage over one that was only able to compete within a certain timeframe. On the other hand, if evolution favored the group over the individual, a population might have an advantage in a population that had its genetic makeup constantly changing, ensuring that it was always able to adapt to environmental changes. However, natural selection generally acts to produce individuals that are more evolutionarily fit, even if it would contradict what would be best for the population. If any individual organism of any species developed a mutation that served no purpose other than to weaken the organism, prevent it from reproducing, and render it more susceptible to injury and disease before eventually killing it, said

organism would be unable to compete with non-mutated members of its species, and that mutation would be quickly wiped out. Furthermore, the aging process is not a very effective way of ensuring that only the most evolutionarily fit individuals remain in a population. Organisms that possessed a disadvantageous mutation would still have to compete with other individuals for resources, and would still have the potential to leave the gene pool if they ever succumbed to injury or disease. On the other hand, any organism that did manage to remain within a population for several generations is likely to be much more likely to possess advantageous genes than any other organism. It is for these reasons that this review will not consider the Programmed Theory of Aging as an accurate theory of aging.

The second main category of theories that attempt to describe the process of aging is the Damage and Error Theory, a category that incorporates theories claiming that the physical symptoms of old age are nothing more than the side-effects of cumulative damage to organisms sustained at the molecular and genetic level. Of course, there are several various explanations that follow this school of thought, with the most well-known being the Free Radical Theory. The theory, first introduced in 1954 (Gerschman et. al 1954), proposed that over time, the cell sustains oxidative damage by extremely reactive chemical metabolites known as reactive oxidative species (ROS). ROS are oxygen-containing metabolic intermediate compounds (including $(O_2^{\cdot-})$, (H_2O_2) , $(LOOH)$, $(HOCl)$, $(ONOO^{\cdot-})$, and (O_3) among others) that are capable of breaking down into free radicals, an extremely reactive family of compounds characterized by the presence of a single unpaired electron within its outer orbit (Li et. al 2016). This unique characteristic makes these chemicals easily capable of causing severe oxidative damage to any macromolecules it comes into contact with, leading to injury within the cell and tissue. However, results collected from several experiments on the impact of ROS on the aging process seem to

conflict with the Free Radical Theory of Aging. Though subjecting an organism to extremely high levels of intracellular free radicals generally results in a quite significant shortening of lifespan compared to that of an unaffected organism (Breitenbach and Eckl 2015), the results of antioxidant tests have not always been perfectly consistent. If the presence of ROS and free radicals was solely responsible for the development of old age in a cell, the presence of antioxidant compounds should reduce the effect of ROS, and therefore increase the organism's lifespan. This is not the case, however, as there exist a significant amount of studies that seem to contradict this idea, with an overexpression of antioxidants within certain systems either having no effect (Mockett et. al 2010) or actually shortening the organism's lifespan (Raamsdonk and Hekimi 2009). In addition, the theory that the oxidative damage caused by high concentrations of ROS is directly responsible for the aging process is contradicted by the fact that molecular oxygen is not necessarily required for aging (Gladyshev 2014). If this theory was true, aging should be greatly reduced in species that live in low-oxygen environments, with organisms in environments completely bereft of oxygen possessing biological immortality. This is not the case, implying that the Free Radical Theory of Aging is incorrect, and that though ROS presence does have a definite influence on the aging process, they are not the primary factor responsible for the onset of old age.

Though the Free Radical Theory of Aging and many theories like it are unable to explain certain experimental results to a fully satisfactory level, and are not at the level necessary to be entirely accepted as valid explanations for the aging process, it remains true that ROS and other biochemical elements have a definite influence on the rate at which many organisms age. The Somatic DNA Damage Theory is a Damage and Error Theory that attempts to reconcile this, stating that the physical effects of aging are the result of a gradual accumulation of damage

sustained by the DNA throughout an organism's lifetime (Kunlin 2010). Through this model, aging gradually manifests itself in an organism once its genetic structure has sustained enough damage to affect the body on a macroscopic level. Though there are likely some controversial experimental results that this theory has yet to explain, for the purposes of this paper, the Somatic DNA Damage Theory will be considered the most accurate explanation of the aging process available so far.

How Do Mutations Occur?

One of the most common methods by which harmful mutations can be introduced to a genetic sequence is through unaddressed replication errors made during DNA synthesis. Occasionally, an incorrect base pair is introduced to the DNA sequence during synthesis, and though the influence of DNA polymerase proofreading processes serves to significantly reduce the rate at which synthesis errors result in actual mutations to a finished strand of DNA, it is not impossible for such a mistake to be made and overlooked by proofreading programs. Alternatively, DNA damage could be induced by external environmental agents, including ionizing radiation, ROS, or any other carcinogenic compounds. In this context, ionizing radiation includes X-rays, gamma rays, or any other kind of radiation powerful enough to cleave electrons from their atoms.

With this much potential for the cell to experience catastrophic DNA damage, the presence of several specialized DNA repair processes is absolutely necessary, as a sufficiently potent mutation could prove deadly to the cell (Collins and Dritschilo 2009). Such repair mechanisms include mismatch repair, in which both the parent and the daughter strand are analyzed alongside one another in order to detect and replace single non-complementary nucleotides, excision repair, in which a faulty portion of the DNA is removed entirely and

replaced via DNA synthesis, or end-joining repair, in which damage that completely cuts both strands of DNA is ameliorated by splicing the two severed ends together (Iyama and Wilson 2014). However, though these repair mechanisms are able to repair most of the damage done to the DNA sequence over time, it is unable to repair all errors, as the rate at which damage occurs is much quicker than the rate of repair mechanisms. In addition, certain DNA-repair systems can be somewhat error-prone themselves, capable of inflicting damage to DNA even as it attempts to repair it (Rodgers and McVey 2016). As a result, over time, such damage will cause an organism to accumulate a growing amount of mutations in the microscopic level that will eventually manifest themselves at the macroscopic level.

Relevance of mtDNA Degradation of the Aging Process

Contrary to earlier traditional views, the aging process is currently believed to not be a process entirely controlled by one specific aging pathway, but by the macroscopic expression of a lifetime's worth of damage sustained by an organism's DNA by any number of biochemical pathways. As a result, efforts are being made to isolate a list of biochemical processes and dysfunctions including telomere attrition, stem cell exhaustion, and cellular senescence that are believed to be at least partially responsible for the development of the aged phenotype (Lopez-Otin et. al 2013). Currently, a significant amount of research is being invested into the influence that mtDNA mutation and subsequent mitochondrial dysfunction has on the aging process.

In eukaryotic organisms, the biochemical processes of cellular respiration, ATP production, and initiation of apoptosis, programmed cell death, is managed by mitochondria, the powerhouse of the cell (Friedman and Nunnari 2014). While it serves as a eukaryotic organelle now, the mitochondria found its place within the cells of eukaryotic organisms roughly 2 billion years ago with the endosymbiotic engulfment of a single proteobacterium by the precursor of the

modern eukaryotic cell (Gabaldon and Huynen 2004). However, though the majority of the original structure and form of the absorbed proteobacterium has either been lost or assimilated by the nuclear genome, modern-day mitochondria have managed to retain a few of their original characteristics, including a double membrane, their means of ATP production, and a somewhat diminished portion of their original genome.

When the effects of ROS damage to the mtDNA were first studied, researchers at the time believed that due to a combination of environmental and organizational factors, the mtDNA was much more vulnerable to mutation than nuclear DNA (nDNA). Though current research has revealed that the mitochondria contains its own set of defenses unique from those employed by the nDNA, the mtDNA is still viewed as being much more sensitive to age-related damage. It has been well documented that condensed, tightly-coiled areas of the nDNA protected by histones are far more resistant to the deleterious effects of DNA-damaging agents, ionizing radiation, and reactive oxidative species (Ljungman and Hanawalt 1992). The nucleus contains a much larger complex of 3 billion base pairs compared to the total length of mtDNA, 16.5 thousand bp (Quiros et. al 2017). Because of this, random mutations are believed to be more devastating if suffered by the mtDNA, as the mitochondria's genome was viewed as being quite primitive, being both incapable of properly defending itself from attack and more likely to be severely damaged by any one mutation. Furthermore, mtDNA is held in dangerously close proximity to the electron transport chain (ETC), the cellular pathway responsible not only for the production of the vast majority of cellular energy, but also for the generation of ROS (Liu et. al 2002). Any damage sustained by the mtDNA has the potential to compromise the effectiveness of the ETC, which itself could produce ROS, further weakening the mtDNA, and resulting in a cascading, self-destructive cataclysm. Due to this effect, though mtDNA remains susceptible to ionizing

radiation or replicative damage or any other means of inflicting radiation, the majority of mtDNA mutation is caused by oxidative damage from ROS. As a result, anti-aging studies have viewed the deterioration of mtDNA as being vitally influential to the overall state of an organism, even when compared to similar insults suffered by the nDNA.

Method

To answer whether mtDNA mutation was responsible for aging, and to determine whether there existed methods capable of artificially restoring normal function to damaged mitochondria, a systematic analysis of the results of existing, relevant experiments was performed in order to gather information necessary to provide an accurate review of the literature. Articles that were considered appropriate for this study were limited to those that analysed the effects of mitochondrial dysfunction on a macroscopic scale, detailed experiments designed to either: address the idea that mitochondrial dysfunction significantly affected the aging process or provide methods by which such dysfunction could be controlled, or provided necessary background information on relevant topics. Databases including Google Scholar, PubMed, and the Pace University Digital Library were utilized in a comprehensive search of peer reviewed journals, based on key terms including “mtDNA”, “anti-aging”, “dysfunction”, and “downregulation”. Further relevant articles were discovered in addition to those located in database searches in the reference sections of original articles. Papers that were included include publications from Aging Cell, Biology Reviews, Cell, The Encyclopedia of Health and Aging, and the United States Census Bureau

Mitochondrial Function is Naturally Damaged in Aged Individuals

If one wishes to test whether or not gradual degradation of the mtDNA is responsible for the aging process, the first obstacle that must be overcome would be to demonstrate that this level of damage naturally and regularly occurs in healthy organisms once they begin to reach old age. If the hypothesis is correct, and mtDNA damage is a significant cause of aging, then one would expect mitochondria within the cells of aged organisms to be far more damaged than those taken from young organisms. To be more specific, not only would the number of mitochondria within a senescent cell be expected to be significantly lower than those of a young cell, but mtDNA taken from older organisms should be significantly damaged, and as a result, overall mitochondrial function should be less effective. If these conditions are not met, and there is no correlation at all between the age of an organism and the effectiveness of its mitochondria, there would be no reason to believe that the aging process is at all affected by the integrity of the mitochondria.

One of the more well-known symptoms of old age is a general decline in the strength, mass, and aerobic capacity of the skeletal muscle, along with other structural and functional changes, long-term alterations within the body that are believed to be the result of an inability to supply muscle cells with adequate energy (Short and Nair 1999). Such a decline in muscle function is present in a wide range of animals, and is believed to be the result of widespread mitochondrial dysfunction. Further observations of the mitochondria only supported this idea, as studies from as far back as 1968 reported significant physical changes in both the size and the number of mitochondria in aged subjects (Tauchi and Sato 1968). An analysis of electron micrographs taken from human liver cells of Japanese individuals aged 21-79 showed an overall decrease in the number of mitochondria with age) along with a corresponding increase in average size, with sharp curves in both slopes as individuals approach year 60. Interestingly, these

mitochondria are not swollen or inflamed, but are instead conglomerates of daughter cells that have simply been unable to complete division. However, not only does aging affect mitochondrial reproductive rate and average density, but also reduces the effectiveness of any mitochondria that remain within the cell (Short et. al 2005). On average, mitochondrial function within a cell experiences a definite age-related decline with both the mitochondrial ATP production rate (MAPR) per gram of muscle and the maximal aerobic capacity (VO_2max) decreasing at a rate of about 8% per decade, along with a general decrease in the amount of intact mtDNA in the muscle that appeared to be very closely correlated with the decline in MAPR and VO_2max . The age-related reduction in intact functioning mtDNA has more than likely had a negative consequence on maximal ATP production and as a result, lowers physical function.

The long-term effects of mitochondrial dysfunction are not restricted to its effects on the skeletal muscle, as all cell types with a high demand for energy, including those found within the muscular, liver, heart, and CNS systems, are hindered by any deterioration in the effectiveness of the mitochondria (Byrne et. al 1991). Any shortage in the level of energy produced through oxidative phosphorylation (OXPHOS) could prove fatal to these cells. The brain of an aged individual is especially at risk for this type of OXPHOS energy shortage, as studies show that the activity of the enzyme cytochrome c oxidase - an enzyme integral to the proper function of the ETC - severely decreases in the frontal cortex, superior temporal cortex, cerebellum, and putamen of the brains of older individuals (Ojaimi et. al 1999). Such a severe decline in the activity of such a crucial enzyme would strongly suggest a corresponding decline in the level of ATP available for use, contributing to the development of age-related neurodegenerative diseases.

Though it may be easy to imagine this damage as only affecting elderly individuals, the mitochondria is constantly suffering from damage throughout an organism's lifespan, though it may only manifest itself on a macroscopic scale towards the later end of an individual's life. Using PCR analysis, mtDNA taken from the heart and brain tissue of adult cadavers was compared with tissue taken from fetal cadavers, revealing that adult tissue contained low levels of a 5 kb mtDNA deletion that, in higher levels, results in the neuromuscular diseases Kearns-Sayre Syndrome (KSS) and progressive external ophthalmoplegia (PEO) (Cortopassi and Arnheim 1990). Such deletions were not found within fetal tissue. Such deletions are not by any means rare, however, as experiments testing the prevalence of the relatively common deletion mtDNA 4977 revealed that the average rate of mutation within the frontal lobe goes from 1 in 1600 in a twenty year old to 1 in 38 to an eighty year old. Situations are more extreme in the temporal lobe, which goes from approximately 1 in 15 thousand in a 20 year old to 1 in 29 in a 90 year old.

Artificial Impairment of Mitochondrial Function Rapidly Accelerates Aging in Affected Organisms

Though data seems to support the idea that there is a correlation between the age of an organism and the effectiveness of its mitochondria, this evidence is not enough to conclude that the former is influenced by the latter. The idea that mtDNA damage is responsible for the development of age would be just as valid as one that argues that mtDNA damage is just another consequence of old age, or even the idea that the correlation between the two conditions is purely coincidental, and that there is no deeper relationship. In order to prove that the aging process is directly affected by mtDNA damage, otherwise healthy organisms that have suffered mtDNA damage or have otherwise had their mtDNA inhibited should rapidly develop symptoms that

strongly mimic what one would observe in a naturally aged organism. In other words, if the hypothesis is correct, then organisms with DNA that is naturally impaired should experience a significantly accelerated rate of aging. However, any tests of this nature that are performed should ensure that affected organisms are in fact suffering from an accelerated aging, and are not simply suffering from an unrelated condition with similar symptoms. Not only should affected organisms have an average life expectancy much shorter than that of the wild-type, and not only should records measure whether organisms have greying hair or wrinkled skin, but the physical and mental abilities of affected organisms should be measured and compared to that of naturally aged organisms. Furthermore, the rates at which age-accelerated organisms develop age-related diseases should be taken into account. In order to mitigate the effect of confounding factors as much as possible, all experiments discussed in this section should utilize methods that only impair mitochondrial function.

DNA polymerase is an enzyme capable of synthesizing DNA from component deoxyribonucleotide building blocks, and is responsible for accurately and effectively replicating the genome and maintaining the integrity of the cell's genetic code. Due to the vital role that this enzyme plays in the maintenance of genetic information of each living organism on the planet, along with the high level of variation among such organisms, multiple specialized variants of DNA Polymerase exist, each operating to maintain and replicate the genetic material of their organism (Garcia-Diaz and Bebenek 2007). One such variant is DNA Polymerase Gamma, the only DNA polymerase capable of operating within animal mitochondria, and as a result, is solely responsible for the replication, proofreading, and repair of the mtDNA (Kaguni 2004). Due to the enzyme's critical role in the maintenance of mitochondrial DNA, DNA Polymerase Gamma has

proven to be a popular target for researchers aiming to test the impact of non-functional mitochondria on animal aging.

If the aging process is a consequence of mitochondrial dysfunction which is itself merely a manifestation of internal mutations and errors within the mtDNA, then organisms that possess mitochondria that are more prone to error are likely to age faster than those that possess normal mitochondria. As a result, studies aiming to test whether or not there is a causative effect between aging and mtDNA mutations tend to utilize a special breed of genetically engineered lab mouse known as *D257A* (Trifunovic et. al 2004). These mice are distinguished from the wild-type by a small two-base substitution in the gene responsible for the production of polymerase gamma, resulting in a final phenotype characterized by a severely defective proofreading unit virtually unable to protect the mtDNA from any number of random mutations, resulting in a mtDNA that sustains a great deal of mutational and oxidative damage in a short period of time (Kujoth et. al 2005). Generally, *D257A* mice are relatively healthy within the first few weeks, but rapidly fell into a premature aged phenotype by week 25 when test animals began to express alopecia (hair loss), graying, and kyphosis (hunched curvature of the spine). Further observation of *D257A* revealed the very early onset of several other age related diseases including presbycusis (age-related hearing loss), loss of bone mass and mineral density, profound reduction in fertility, and heart disease. By the end of the study, the average lifespan of *D257A* mice was approximately 416 days, compared to the average lifespan of the wild type, approximately 850 days. If this dramatically altered phenotype can be considered aging, then studies on *D257A* mice strongly suggest that mtDNA damage is responsible for inducing aging, and that it can operate independently of any change in ROS or any other system. Furthermore, similar research into transgenic variants of *D257A* mice capable of inducing full-body depletion of the mtDNA once it

ingested the compound doxycycline (dox) yielded similar results, though symptoms expressed by this organism seemed to be limited to skin inflammation, hair loss, and decrease in average lifespan (Singh et. al 2018). However, affected organisms that were taken off of doxycycline and allowed to replenish their mtDNA returned to a physical state very closely resembling that of the wild-type.

Reversing or Inhibiting mtDNA Damage Can Delay the Onset of Old Age

If mtDNA damage, increased ROS production, and decline in the general effectiveness of the organism's mitochondria played a major role in the onset of old age, then one can expect any technique that prevents or repairs mtDNA damage and inhibits the ROS cascading effect to have a positive effect on affected organisms. In other words, any organism that has been treated to have their mtDNA artificially restored or protected from damage should experience a rate of aging far more delayed than that of their unaffected counterparts. If not, then that would imply that though mtDNA damage plays a significant role in the rate at which an organism ages, the aging process cannot be solely decided by the functionality of an organism's mitochondria, and mtDNA damage simply triggers one or more runaway effects that can operate independently. Furthermore, data collected from these tests should not only illustrate whether or not this treatment increased the average lifespan of treated organisms, but also consider whether the onset of age-related diseases and conditions is similarly affected.

If a buildup of oxidative stress caused by defects in mitochondria generating toxic ROS causes the fatigue, muscle atrophy, and skeletal muscle dysfunction commonly observed in elderly individuals, then a treatment that merely relieves oxidative stress should be able to yield a definite decline in age-related muscular atrophy. The mitochondrial peptide SS-31, capable of both directly inhibiting the production of ROS as well as scavenging and destroying existing

ROS has been used to test this hypothesis (Szeto et. al 2011). Mitochondria taken from the skeletal muscle of aged mice treated with a single injection of SS-31 showed a very strong reversal of any age-related decline in the rates of both ATP production and oxidative phosphorylation, with tests of physical ability very closely resembling the average physical capacities of young mice. Repeating this experiment with young mice did not mimic earlier results, as measurements of the muscular capabilities of both treated and untreated young mice were nearly identical. This seems to indicate that SS-31 is not simply a general performance enhancer, but selectively reverses the decline in muscular function brought about as a consequence of aging (Siegel et. al 2013).

Cellular levels of carnitine, a compound responsible for transporting digested elements of fatty acids into the mitochondria for conversion into ATP, significantly decrease as a function of age. As a result, this prompted similar experiments in which the compound acetyl-l-carnitine (ALCAR) was given to aged mice as a dietary supplement in order to test whether it could counteract age-based decline in cells. Though ALCAR supplementation failed to increase life expectancy or result in any immediately noticeable physical changes, aged mice that did have missing ALCAR replaced via dietary supplement did have cellular oxygen consumption restored to a level resembling the wild-type, along with a corresponding increase in ambulatory activity (Hagen et. al 1998).

Of course, while experiments displaying an improvement in cellular function and overall increased quality of life for affected individuals may be promising, if one wishes to gauge whether it is possible to inhibit aging through the mitochondria, they would most likely be interested in an increase in lifespan over any other effect. Test mice that were genetically altered to overexpress catalase, the enzyme used to decompose the ROS hydrogen peroxide into water

and molecular oxygen, in the mitochondria (MCAT), nucleus (NCAT), and peroxisome (PCAT) all expressed a noticeable increase in average life span. However, maintained observation of genetically altered litters revealed that both the average and maximum lifespans were most significantly affected in MCAT mice with an increase of 5 months and 5.5 months, respectively. The overexpression of catalase within the mitochondria itself prevented ROS such as hydrogen peroxide from damaging the mtDNA, and strongly aided in the inhibition of oxidative stress (Schriner et. al 2005). Follow-up observations revealed no noticeable detrimental side-effects to this process, though studies are still going.

Conclusion

Though there do exist several means by which aging is, if not prevented, at least postponed in model organisms, such techniques only manage to extend lifespan for a few months, if at all. Furthermore, the future of anti-aging research along these lines is uncertain, as the ultimate success of age-prevention technology centered around the maintenance of mtDNA is linked to the accuracy of the mitochondrial theory as a realistic model of the aging process. Though there have been several experiments that seem to confirm the hypothesis that reactive oxidative species caused by mtDNA damage is the primary cause of aging, there is no reason to assume that the theory is not just an early, unrefined version of a future, more accurate explanation, or that mitochondrial dysfunction isn't

merely just a single aspect of a much more complex, multi-faceted aging process. Though there are several papers outlining possible flaws in the mitochondrial theory of aging that have yet to be answered to a satisfactory degree (Rasmussen et. al 2003), one of the main flaws in the concept of mitochondrial damage as the single primary factor in the aging process can be found in progeroid diseases, defined as a grouping of rare genetic disorders that seem to mimic accelerated physiological aging. If a damaged mitochondrial genome was the main factor contributing towards the onset of old age, one would expect every progeroid disease to involve severe damage to the mitochondria. However, several progeroid diseases, including Werner syndrome and Cockayne syndrome, do not seem to involve any mitochondrial damage at all.

Any future studies done in this topic would involve a study of any future negative side effects, either physical or mental, experienced by organisms that have undergone procedures that have granted them an extension of their lifespan. Emphasis would be placed on whether side-effects are caused by the treatment itself, or are the animal's general behavioral reactions to being restored to a superficially younger state.

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