Hypotheses on the extended phenotype of the mitochondrion: sex, mortality, and aging

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Abstract

How did sex evolve, how is sex evolutionary stable, why do eukaryotes appear mortal, and why do eukaryotes age, are all pressing questions. This paper presents a mitochondrial perspective on the evolution of the eukaryotic cell that appears capable of answering these questions. Rather than viewing a mitochondrion as a passive entity taken up by an archaeal host that remains in the driving seat, mitochondria are viewed as the key force driving eukaryogenesis. The proto-mitochondrion is presumed to have manipulated its archaeal host to engage in sex in order to replicate itself in a more and more beneficial environment. This process is hypothesized to still be operating today as a result of the mitochondrion's continued production of reactive oxygen species (ROS). The specific production of ROS by the mitochondrion appears to be an intentional mechanism to cause the organism to age, and ultimately to die. Faced with mortality, if the organism wishes to pass on its nuclear genes it will typically engage in sex as a means of resetting age. Eukaryotic species that instead reproduced parthenogenetically would find themselves out-competed by sexual species due to the reassortment of genes that comes with sex. The mitochondrial genome benefits via kin selection from the death of its host as a result of an increased ability to adapt to a changing environment that comes from a shortened time between successive sexual generations. The resulting model appears capable of explaining the seeming intentionality of many age-related diseases, and provides a high level theoretical framework for better understanding them.

Keywords: evolution, eukaryogenesis, extended phenotype, mitochondria, sex, mortality, aging, reactive oxygen species, cellular senescence, age-related diseases.

1 Introduction

This paper offers a mitochondrial perspective of the evolution of the eukaryotic cell. It is not that the nuclear genome is unimportant, but that it might be over-emphasized, and that by giving due consideration to the role of the mitochondrion a clearer picture of eukaryogenesis, sex, mortality, and aging emerges.

Many age-related diseases appear to be caused by cellular senescence, which appears to be activated by telomeric DNA damage, which in turn appears to be caused by reactive oxygen species (ROS).

The core idea of this paper is that the production of ROS by the mitochondria can be viewed as an intentional mechanism by the mitochondrial genome to cause the individual organism to die. A shortened lifespan will reduce the mean time between successive sexual generations, and thus increase the ability of the population to adapt to a changing environment. The mitochondrial genome benefits from this increased ability to adapt via kin selection.

Syngamy is the eukaryotic process that produces one diploid cell from two haploid cells. Meiosis is the process that produces four haploid cells from two diploid cells. As used in this paper, sex refers to the combination of syngamy and meiosis. Sex can be viewed as a mechanism by which haploid cells produce new haploid cells, or alternatively the process by which diploid cells produce new diploid cells.

The existence of sex is troubling to some biologists because of the two-fold cost it imposes; that is only half of a parent's alleles get passed on to each offspring[1]. Parthenogenetic reproduction has no such constraint. All of the parent's alleles get passed on to each offspring. This raises the question of how sex might have evolved, and how it might continue to exist, when the alleles for it would seem hellbent on their own demise.

Mortality is also troubling. Evolution appears able to produce a myriad of complex organismal forms, but unable to perform the seemingly much simpler task of keeping them working. The fact that two relatively recently diverged species, such as mice and men, have such widely different lifespans suggests mortality may be deliberate. But why and how?

The phrase "the extended phenotype" was developed by Richard Dawkins to refer to phenotypic effects beyond the boundary of the organism[2]. For example, the extended phenotype of the beaver includes the dams it builds. The extended phenotype of the mitochondrion is the phenotypic effects of the mitochondrion beyond the outer mitochondrial membrane. This paper considers extended phenotypic features of the mitochondrion, and in particular a role in the evolution of sex, mortality, and aging.

The eukaryotic cell is believed to have evolved from a symbiotic relationship between an archaeon and an alphaproteobacterion[3]. Most attempts to understand the eukaryotic cell focus on the nuclear chromosomes, treating the incorporation of the mitochondrion as an energy providing afterthought. In terms of size the mitochondrial genome is small, but in terms of what it brings to the equation, a 15-fold increase in ATP[4], it is large, and thus it should have been expected to play a major role in the evolution of the eukaryotic cell. In addition, the mitochondrial genome may be small today, but historically the proto-mitochondrial genome is likely to have been much larger. Alphaproteobacterial spotted fever group Rickettsia genomes are around 1.3M base pairs.

Hypothesis 1: Sex evolved as a means for the proto-mitochondrion to increase the fitness of its host environment.

Hypothesis 2: Sex continues to be the means by which the mitochondrial genome increases the fitness of its host environment.

Hypothesis 3: By enforcing mortality, the mitochondrial genome forces the nuclear genome to engage in frequent sexual recombination.

Hypothesis 4: Aging is a process in which the mitochondrial genome enforces mortality.

Hypothesis 5: Pro-aging nuclear genes will also exhibit some vital life-enhancing function.

Corollary 5.1: Anti-aging interventions can commonly be expected to exhibit reduced biological fitness in the evolutionary environment.

Hypothesis 6: ROS production is the fundamental mechanism by which the mitochondrial genome causes aging.

Hypothesis 7: Cellular senescence is a key downstream mechanism of aging in vertebrates.

Table 1: Hypotheses on the extended phenotype of the mitochondrion

And known Asgard archaeal genomes, putative eukaryotic ancestors[5], aren't a lot larger, ranging from 1.4-5.7M base pairs. In short, the proto-mitochondrial genome had a lot of bargaining power over the nature of the union.

After briefly reviewing some basic biological concepts and framing the problem, the bulk of this paper develops a series of seven related hypotheses on the extended phenotype of the mitochondrion (HEPM). These hypotheses lay the foundation for, and formalize, the core idea that through kin selection the mitochondria cause the individual host organism to die so as to maximize the fitness of their host environment. These hypotheses along with one corollary are shown in Table 1. As the paper develops, the predictions of these hypotheses are compared to observations. Once the hypotheses are fully developed the conflict that results from the different reproductive mechanisms of the nuclear and mitochondrial genomes is investigated. This is followed by an exploration of the implications of the hypotheses for addressing many age-related diseases. Finally, in the discussion section, an assessment of the evidence for and against the hypotheses is weighed.

1.1 Mitochondria

Mitochondria are reviewed in detail in Appendix A. Only the role of mitochondria in apoptosis and senescence is reviewed here.

Mitochondria play a key role in apoptosis[6]. The pathways leading to apoptosis all flow through the mitochondria, and the release of apoptotic inter-mitochondrial membrane proteins into the

cytosol irreversibly starts the apoptotic process[7]. H_2O_2 oxidizes cardiolipin found in the inner membrane causing it to release bound cytochrome c[8]. Oxidized cardiolipin also helps open the mitochondrial permeability transition pore in the outer membrane[9, 10, 11]. Opening of the pore leads to a swelling of the matrix, rupturing the outer mitochondrial membrane, and the release of apoptotic intermembrane proteins into the cytosol including cytochrome c[12]. The reason why mitochondria play such a key role in apoptosis presently appears to be unknown.

Mitochondria also play a key role in cellular senescence, which is a state of seemingly permanent growth arrest of a cell. In cellular senescence mitochondria are enlarged, elongated, and hyperfused[13]. Moreover they are less efficient at producing ATP, with a decrease in the mitochondrial membrane potential, and an increase in the production of reactive oxygen species (ROS)[13]. Mitochondrial ROS may cause DNA damage that leads to telomere shortening in replicative cellular senescence[13]. Senescent cells in which the mitochondria have been eliminated are capable of surviving via glycolysis[14]. Such cells remain at cell cycle arrest, but are unable to generate a senescence-associated secretory phenotype[14]. The reason why mitochondria play such an important role in senescence appears unknown.

1.2 Evolution of the eukaryotic cell

Alphaproteobacteria are a class of bacteria. The eukaryotic cell is widely believed to have evolved from a symbiotic relationship between an archaeon and an alphaproteobacterion, with the alphaproteobacterion becoming the mitochondrial organelle[3]. Much of the original DNA of the alphaproteobacteria is believed to have relocated to the nucleus, leaving behind only a small mtDNA remnant.

Exactly how an alphaproteobacteria ended up living inside an archaeon isn't certain. There seem to be few theories capable of explaining the mechanics of how eukaryotes originally evolved. Currently the only serious attempt to explain the mechanism of evolution of the eukaryotes appears to be the viral eukaryogenesis hypothesis.

The viral eukaryogenesis hypothesis posits that the eukaryotic cell evolved from a virus, archaeon, and alphaproteobacteria[15]. Difficulties with the viral eukaryogenesis hypothesis include viruses would need to evolve the means to replicate by themselves if they are to eventually evolve into gametophytes, and the lack of any known double stranded DNA viruses with segmented genomes; so the theory doesn't explain the origin of chromosomes.

An alternative line of reasoning starts with the archaeon's development of the cytoskeleton component actin[16, 17, 18]. Actin filaments would have allowed the archaeon to extend its plasma membrane to engulf large particles, which it would then attempt to digest, leading to the development of phagocytosis[19]. When that ingested particle happened to be a living cell, the ingested cell would evolve in such a way as to attempt to resist the full phagocytic effects of ingestion. An example of this is provided by *Rickettsia conorii*, an intracellular pathogen, which enters the host by inducing host phagocytosis, and then escapes from the phagosome into the host's cytosol[20]. It should be noted that Rickettsia is a genus of alphaproteobacteria.

Once inside the archaeon the alphaproteobacteria would have had three possible ways to propagate. Similar to the lytic or lysogenic cycles of bacteriophages and viruses, it could replicate until the

host cell bursts and then find new host cells to infect, or it could attempt to ensure that it is faithfully propagated to each descendant of the host cell. Additionally the alphaproteobacteria could use actin based motility to spread from cell to cell. An example of this is again provided by the Rickettsia. The typhus group Rickettsia cause host cell lysis, while the spotted fever group spread from cell to cell by means of actin filaments[21]. It is worth noting that spotted fever group Rickettsia infection doesn't necessarily lead to host cell death, because avirulent strains of the spotted fever group exist that are capable of coexisting with their host in what might be described as a parasitic endosymbiosis[21].

The initially defenseless host might be expected to evolve defenses against the alphaproteobacterial parasites. These defenses are unlikely to be complete, we are still vulnerable to Rickettsia today, but are likely to be substantial. The exception being if the endosymbiont provides a benefit to the host. This proved to be the case with the mitochondria, which through oxidative phosphorylation increases the ATP available to the host by roughly a massive 15 fold over the glycolysis of anaerobic fermentation[22]. This then leads to a mostly cooperative relationship between the host and parasite. Over time the endosymbiont lost the ability to survive outside of the host.

1.3 Group selection and kin selection

Group selection is the hypothesis that natural selection acts for the good of a group, such as a species. Group selection is widely dismissed by evolutionary biologists[23]. Kin selection is the hypothesis that evolution is capable of acting not just for the benefit of an organism and its offspring, but also for the benefit of the organism's relatives. Kin selection is widely accepted by evolutionary biologists[24].

1.4 Active germline replicators

An active germline replicator is an entity of which copies can be made and whose nature has some influence over the probability of it being copied[2].

Both the archaeal host and the alphaproteobacterial proto-mitochondrion are active germline replicators. The germ cells and zygotes of eukaryotes might be described as low fidelity active germline replicators. Germ cells and zygotes make copies of themselves in an environment made up of other germ cells and zygotes. The strategy they employ may involve creating zygotes, germ cells, somatic cells, and multicellular organisms, but in the end they produce more copies of themselves. They are low fidelity replicators in the sense that the DNA sequences of the copies only partially reflect the original due to homologous recombination of allelic sequences.

To be pedantic, the DNA should probably be viewed as forming the active germline replicator, and the archaeon or proto-mitochondria is just a vehicle for the replicator, but it is often easier to speak in terms of replicating proto-mitochondria than to spell out every time that it is the DNA of the replicating proto-mitochondria that is the replicator, with the rest of the mitochondrion existing because it assists in making copies of the replicator.

An important question in biology is: how can active germline replicators (the archaea and alphaproteobacteria) combine to make other active germline replicators (eukaryotes). This is the theme of

the rest of this paper.

1.5 The problem of sex

For species in changing environments sex typically offers large fitness advantages, but at the nuclear genetic level it is difficult to understand how it evolved, and why it continues to exist.

Reported advantages of sex include the ability to combine the best mutations from several organisms, resistance to parasites, clearance of deleterious mutations, and an increase in the speed of evolution[25]. These are all advantages for the individual or the species. But evolution is widely viewed as not working for the good of the individual or the species, but for the good of the gene[26].

The fundamental problem with sex is it results in only half of each parents' alleles getting passed on to each offspring. This includes the alleles promoting sex.

Consider a very simple scenario in which a single dominant nuclear allele for parthenogenesis arises in a large sexual population. At the population steady state each sexual female produces an average of two offspring; only one of which contains a given parent's allele. Meanwhile, assuming the male parental investment in the sexual case is zero, the asexual organisms will also produce two offspring, doubling the population of the allele. Consequently the allele for parthenogenesis should rapidly increase in the population and allele for sex should rapidly be lost from the population. This is J. Maynard Smith's classic argument for the two-fold cost of sex[1].

Care must be taken when using the phrase "two-fold cost". In the literature "two-fold cost" sometimes refers to gene dilution, as above, and sometimes it refers to the cost of producing males[27]. For gene dilution, extra care must be taken with the phrase "two-fold cost" as it is not something that gets offset against the benefits of sex for a population. Rather it is a cost born by nuclear alleles in favor of sex. Even when sex is highly beneficial to the population, the two fold-cost can make nuclear alleles in favor of sex become extinct. It is true that parthenogenetic reproduction is associated with a small, gradual, loss in fitness, but this doesn't come close to the two-fold cost over the time frame in which nuclear alleles become extinct. The cost of producing males is something that does get offset against the benefits of sex for a population, making genome dilution the more fundamental problem.

A more complex scenario than the one just considered, in which there are multiple dominant or recessive alleles for sex or for parthenogenesis, is unlikely to change the two-fold cost. The problem with sex lies with the fundamental nuclear genome sharing nature of sex, not with the genes promoting it. So how might sex have evolved, and how might it continue to exist?

Smith's argument depends critically on the amount of male parental investment. If male parental investment was 50% of the total parental investment in the offspring, the asexual organism would only produce one offspring. But even if male parental investment is 50% there are still problems with sex. A parthenogenetic reproducer could mimic a female and accept parental investment from a male, but then discard the male's genes. Or the allele for parthenogenesis could favor itself during or post meiosis by killing its siblings. Such an allele might be expected to spread within a population, even if it is harmful to the success of the species. Fundamentally, sex involves an allele sharing half of its accrued rewards with an unknown competitor. This does not seem a productive

thing to do.

Despite the advantages of sex for a species, the two-fold cost to alleles in favor of sex, and hence Darwinian evolution, seem to argue against it.

2 Sex

2.1 A proposed evolution of sex

It is proposed here that sex evolved as a means for mitochondrial active germline replicators to replicate themselves inside of more and more suitable hosts. The mitochondria were engaged in the ultimate selective breeding experiment, crossbreeding those host nuclear chromosomes that proved successful in previous generations.

> Hypothesis 1: Sex evolved as a means for the proto-mitochondrion to increase the fitness of its host environment.

The proposed route to sex is as follows. Imagine an actin propelled alphaproteobacteria attempting to spread from cell to cell by punching a hole in two apposed archaeal cells' plasma membranes. This would be similar to the way in which the spotted fever group Rickettsia are known to spread by punching a hole in their host by means of actin, and then entering a neighboring cell[21, 28, 29]. If the plasma membranes were close enough to each other when the holes were punched then there is a possibility that the holes might heal by joining together around the apposing points on their rims. You would then end up with a single cell containing alphaproteobacteria and two copies of the archaeal genome. This would be much like the mitochondria and two copies of the nuclear genome found in eukaryotic cells today. When the cell next replicated the two archaeal genomes would become four genomes, which would be followed by cell division. All it would take is for this to be followed by a second cell division, and you would have something that is starting to resemble syngamy followed by meiosis – minus the important reassortment of nuclear genes. This is shown in Figure 1. Partial reassortment of genes could occur if the circular archaeal genomes were broken into distinct lengths: primitive nuclear chromosomes.

Reassortment of genes would bring huge benefits to the organism. Suddenly evolution can occur in parallel across all the genomes in the population, and the best features of each merged, instead of having to be evolved along a single lineage. Ortholog radA in archaea, recA in bacteria, and Dmc1 in eukaryotes, is a key gene in homologous recombination[30]. It is capable of scanning for double-stranded DNA homologous to a single-stranded DNA template. Since radA and recA are found in archaea and alphaproteobacteria, its recruitment to the process could thus quickly lead to full blown meiosis[30]. At first the process would be messy, with multiple archaeal cells sometimes merging, and chromosomes and proto-mitochondria not getting distributed evenly among descendants. Frequently failures would occur, but the sequence of steps leading to tightly regulated syngamy and meiosis is precisely the sort of thing evolution is good at climbing.

An evolution of sex like the one described would explain why anaerobic environments don't contain any anciently amitochondrial eukaryotic species[31]. The mitochondria was there at the start of

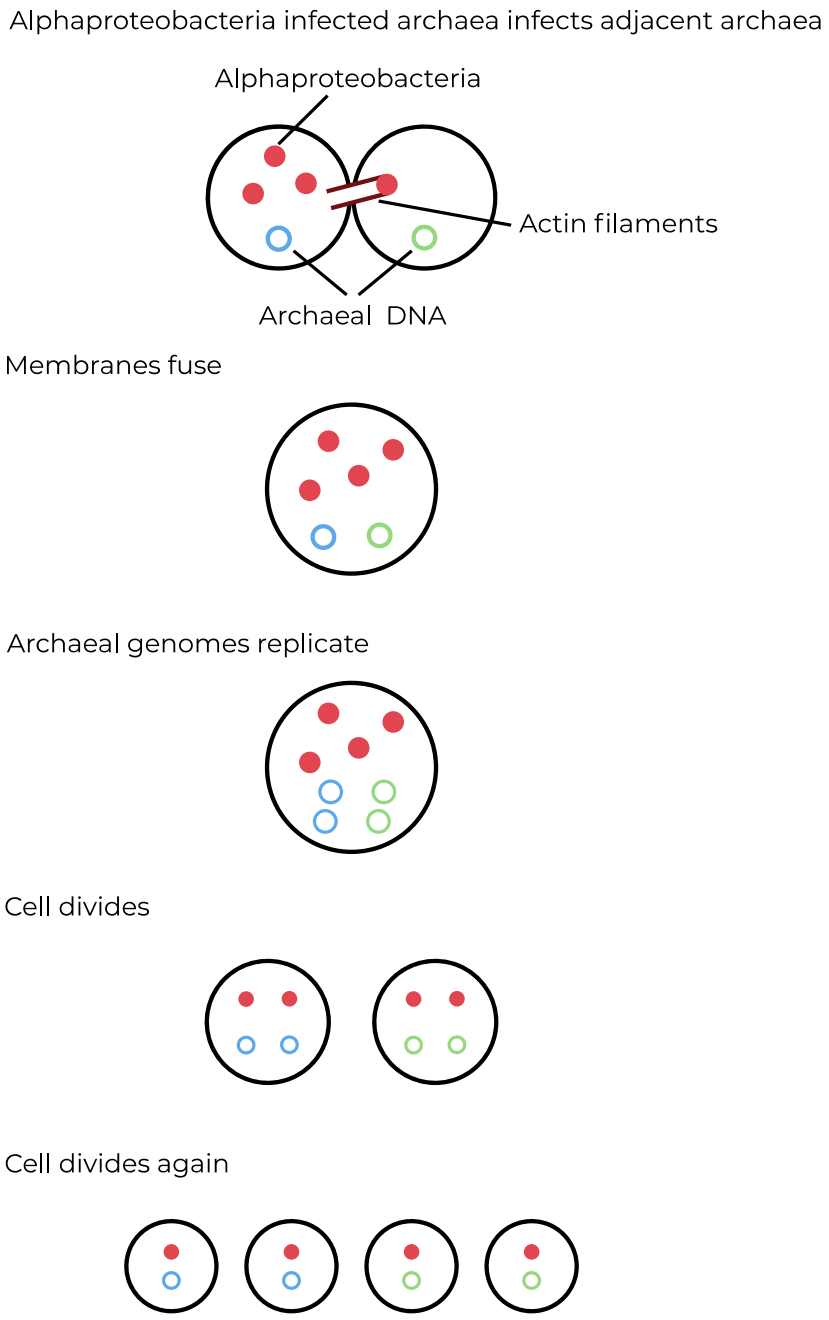

Figure 1: Proposed route to sex. An alphaproteobacteria from an infected archaea spreads to an adjacent archaea by means of actin filaments. The membranes of the two archaeal cells then fuse. The archaea genomes then replicate followed by cell division. If a second round of cell division then occurs the process starts to be reminiscent of syngamy followed by meiosis. The only major missing component is the reassortment of genes.

eukaryogenesis.

The possibility that the alphaproteobacteria that evolved into the mitochondrion had a broad host cell range has the potential to explain difficulty in determining the archaeal ancestor of the eukaryotic cell. There need not be one single ancestor or ancestor species.

An actin-based alphaproteobacteria motility model of eukaryogenesis is a simple theory in which mitochondria replicate themselves inside more and more suitable hosts. Other theories of eukaryogenesis are possible. One such other theory is that sex somehow evolved as a means to provide compatibility between a rapidly mutating mitochondrial genome and a more slowly mutating archaeal genome[32]. Provided at least part of the mitochondrial genome survived the process of crossbreeding nuclear genomes intact, other such theories would lead to similar conclusions regarding the nature of mortality and aging to those to be reached here.

2.2 On sex

Who benefits from sex? As hypothesized in this paper, sex evolved for the benefit of the proto-mitochondrial genome. Proto-mitochondrial genomes were manipulating their environment (the archaeal host) in order to make it more probable they will survive.

Do the nuclear genes of the originally archaeal host benefit from sex? A nuclear gene benefits relative to a nuclear gene in an asexual species by being placed in a fitter and fitter environment. This environment is created by the recombination of the other nuclear genes. But a particular nuclear allele does not benefit relative to some other allele of the same nuclear gene. Each nuclear allele would prefer to reproduce selfishly, asexually. This point is no different than a multi-player prisoner's dilemma. Everyone might benefit from cooperating, but an individual allele gains a strong initial advantage by defecting.

Note that although sex had benefits for the species, it need not. Sex could have been harmful to the success of the species, but so long as the proto-mitochondrial genome benefits, in the short run it would still occur. Depending on how harmful it was this could lead to the decline or extinction of the population. This is in contrast to most existing theories on sex, which attempt to divine how both nuclear alleles and the species benefit[25].

Why did the nuclear genes participate in sex then? This is a thorny question which will be addressed in section 3 dealing with mortality. For now, simply note that the species benefited from the recombination of advantageous alleles and from the energy provided by the mitochondrion: the primary beneficiary of sex.

How does the mitochondrial genome cause sex? Today, this is difficult to see. Most mitochondrial genes that once existed to cause sex, say by riding an actin filament, punching a hole in two cell membranes, and causing recombination to occur, have probably nearly all long since migrated to nuclear genes, and been replaced by other nuclear genes. As will be explored in sections 3 and 4 dealing with mortality and aging, the mitochondrial genome maintains a genetic mechanism that causes sex to occur through the generation of reactive oxygen species.

2.3 Sex in present-day eukaryotes

One possibility is the alphaproteobacterion got the ball rolling with respect to the occurrence of sex, and then the alphaproteobacterion faded into the background becoming the mitochondrion, and the nuclear mechanisms of sex became self supporting. This however fails to explain how sex can continue to exist in unicellular organisms that are capable of parthenogenetic reproduction given the two-fold cost to alleles in favor of sex. Also as explored in section 3, it fails to explain why eukaryotes appear mortal. And it also doesn't explain why the mitochondrion continues to maintain its own separate genome.

The near universality of mitochondria, mitochondrial DNA, and sex in present day eukaryotes allows us to hypothesize that the crossbreeding of nuclear chromosomes by the mitochondrion isn't confined just to eukaryotic evolution, but that it continues to occur today.

> Hypothesis 2: Sex continues to be the means by which the mitochondrial genome increases the fitness of its host environment.

How the tiny mitochondrial genome can achieve this will be explored in sections 3 and 4 dealing with mortality and aging.

2.3.1 Asexual eukaryotes

A nuclear gene that prevents sex and leads to parthenogenetic reproduction might be expected to propagate within a species, but represents an evolutionary dead end. The benefits of sex will be lost, and the species will be out-competed by other species. This is consistent with the observation that it is very rare for a taxon higher than a species to consist entirely of asexual species[33]. The only reported exception appears to be the bdelloid rotifers:

- The bdelloid rotifers are famous as a class of ancient asexual eukaryotes[34]. Bdelloid rotifers appear to engage in interspecies and intraspecies horizontal gene transfer[35, 36]. Importantly bdelloid genomes have been found to contain pairs of homologous chromosomes and engage in occasional gene transfer between the homologous chromosomes[37]. This may go some way to explaining why the bdelloid rotifers don't need to engage in sex. They may get one of the benefits of sex through other means: the ability to try out multiple mutations without permanently losing the original genome.

  There may also be a mitochondrial angle to the bdelloid rotifer story. The bdelloid rotifers *Rotaria rotatoria* and *Philodina citrina* mitochondrial genomes have been sequenced[38, 39]. The large subunit sequence sizes are 529 and 477 nucleotides respectively. The mitochondrial small ribosomal subunit sizes are 521 and 720 nucleotides respectively. This seems very small to be a functioning ribosome, particularly for the large subunit. For comparison, the human large and small subunits are 1,559 and 954 nucleotides. As described in section 7, a review of the mitochondrial large subunit sizes of 13,645 species only turned up 30 sequences that were putatively smaller than that of *R. rotatoria*. All 30 had substantial adjacent non-coding regions, suggesting they were incompletely identified. Thus it is conceivable that bdelloid

rotifers have lost a fully functioning stand-alone mitochondrial ribosomal gene along with the loss of sex, and would then require some nuclear component to assist in the translation of the mitochondrial genome. This might seem unlikely, but so does a large subunit size of 529 or 477 nucleotides.

It should be noted that the non-bdelloid rotifers *Brachionus plicatilis* and *Brachionus rubens* mitochondrial genomes are unusual in that they consist of two chromosomes[40]. Whether this odd arrangement was a stepping stone to the bdelloid's asexuality isn't known. Summarizing, something appears to be going on in the mitochondrial genome of bdelloid rotifers.

Two taxa that were once thought to be asexual are aphids of the genus *Trama*, and bivalved crustaceans of the family Darwinulidae[34]:

- *Trama* was reportedly a genus of asexual aphids, but with the report of sex in *Trama troglodytes*, this is no longer the case[41].
- Like *Trama*, the darwinulid ostracods were long thought to be asexual, but males have now been found in one species, suggesting any loss of sex might have been more recent[42].

If the loss of sex is represents an evolutionary dead end, ancient asexual species should be rare. They are. Besides the bdelloid rotifers and darwinulid ostracods the only other well known ancient asexuals appear to be the arbuscular mycorrhizal fungi[34]:

- Like the bdelloid rotifers, arbuscular mycorrhizal fungi might have found an alternative to sex. In arbuscular mycorrhizal fungi, offspring receive hundreds of nuclei from their parent[43]. Thus there is a population of individually mutating nuclear genomes that might provide some of the benefits of sex seen in other organisms.

If the mitochondrial genome is the cause of sex, it follows sex is more likely to be lost when the mitochondrial genome is delivering little or no value to the resulting organism, such as in anaerobic environments. Here the loss of mitochondria or the loss of the mitochondrial genome might be expected to lead to the loss of sex. It is thus worth considering a few other taxa within which sex appears to have been more recently lost:

- Further evidence that the mitochondrial genome has something to do with sex is provided by the microsporidia. Microsporidia are a group of fungi that lack mitochondria[44]. They do however have an organelle called a mitosome, that appears to have been derived from the mitochondrion[44]. Mitosomes appear to have lost their organellar DNA. This makes the fact that some species of microsporidia are entirely asexual interesting. Even more interesting is the fact that this loss of sex doesn't appear to have occurred in one ancient ancestral lineage, but to have occurred several times in different lineages[45]. This loss of sexuality has occurred in the absence of a mitochondrial genome.
- Diplomonads and trichomonads are two orders that have lost their mitochondria[46]. Despite some diplomonads having genes for meiosis, they are not known to be sexual[47]. Trichomonads are also believed to be asexual[48]. Once again the loss of mitochondria and the possibility for asexuality go hand in hand.

In conclusion there seems to be a relationship between missing or unusual mitochondrial systems and asexuality in eukaryotes. More specifically, asexuality rarely transcends taxa larger than a species, asexuality frequently appears to be an evolutionary dead end with very few ancient asexuals, and asexuality is particularly common in amitochondrial species.

2.4 Sex and species

In a seemingly strange coincidence, DNA barcodes of the mitochondrial COX1 gene make it possible to group organisms into clusters, and these clusters usually just happen to be the same as domain expert's judgments of distinct species[49, 50]. This happens even though new species are believed to most commonly be formed through vicariant allopatric speciation[51], in which there isn't a strong population bottleneck on the mitochondrial genome.

There are many definitions for the concept of a species. The mitochondrial perspective provides another one. A species could be defined as the phenotypes of all of the nuclear genomes associated with a set of closely related kin mitochondrial genomes. The mitochondrial genomes are in competition with other mitochondrial genomes, but they cooperate with kin, and they benefit from the crossbreeding of the nuclear genomes associated with kin mitochondrial genomes. The larger the nuclear genome pool, the more opportunities the mitochondrial genomes and thus the species have to survive. Cooperation between kin mitochondria has the power to explain why mitochondrial DNA barcodes should cluster organisms into species.

Mitochondrial genomes function as selfish replicators, much like prokaryote genomes, but within a more complex environment. The nuclear genome pool forms the extended environment within which related mitochondrial genomes evolve, assisting some mitochondrial genomes, and impinging on others, and generally ensuring all the mitochondrial genomes associated with a nuclear genome pool remain sufficiently similar, that is, related.

In this environment, uniparental inheritance of mitochondrial genomes may result from, by analogy with Fisher's fundamental theorem of natural selection[52], uniparental inheritance delivering a greater variance in mitochondrial genome associated fitness than biparental inheritance. It is from this variance in fitness that selection drives increased fitness. This advantage for uniparental mitochondrial inheritance has been demonstrated in silico[53].

If sex was lost in some species this would reduce the fitness for the embedded mitochondrial genomes relative to the mitochondrial genomes of other species as they would no longer benefit from the crossbreeding of the nuclear genomes. It would represent a rapid evolutionary dead end for the species.

3 Mortality

So far we have seen how the mitochondrial genome might favor sex so that it gets to exist in a fitter and fitter environment. The theory we have developed so far however is incomplete. Nuclear alleles in favor of sex might be expected to be replaced by alleles favoring parthenogenetic reproduction. Or when parthenogenetic reproduction is not a possible option, nuclear alleles in favor of sex might

be expected to be replaced by alleles favoring continued mitotic growth. In order to understand why this doesn't occur it will be necessary to examine eukaryotic mortality, and in particular to hypothesize a role for the mitochondrial genome in bringing about eukaryotic mortality. How mortality promotes sex will be explained below.

Eukaryotic mortality refers to the existence of an apparent intrinsic time limit for which a eukaryotic organism can live, before death occurs. This time limit may be measured in terms of actual time, aggregate metabolic inputs, or some other organismal process. Here an organism that only dies as a result of extrinsic evolutionarily unavoidable misfortune is not viewed as being mortal.

The nuclear alleles desire immortality and asexual reproduction. Suppose after a certain time period without sex the mitochondria always sabotaged the existence of the organism. If this was the case, the nuclear alleles would have no alternative way to continue to exist other than to periodically engage in sex. This is precisely what appears to happen. Individual eukaryotes age and die, the mitochondrion appears to be implicated in organism mortality, and reproduction causes a resetting of the aging process.

Hypothesis 3: By enforcing mortality, the mitochondrial genome forces the nuclear genome to engage in frequent sexual recombination.

For single celled organisms, mortality might only come after several rounds of parthenogenetic reproduction. Sexual recombination, not parthenogenetic reproduction, is expected to reset the age of the organism.

For multicellular organisms, mortality means that the strategy of surviving by just continuously growing through mitosis is ultimately going to fail. If the nuclear genome is going to survive it ultimately has to engage in meiosis.

How mitochondria bring about mortality will be explored in section 4. For now it is sufficient to recall that mitochondrial reactive oxygen species (ROS) are harmful to many cellular components, and that the mitochondria play key roles in apoptosis and cellular senescence.

Death as a means of promoting a fitness increase that comes from sex is capable of explaining why unicellular eukaryotes are capable of committing apoptosis[54].

That the population will benefit from a shorter generation time than desired by the nuclear alleles may appear fairly obvious. Sex provides an increase in fitness as a result of the combination of advantageous alleles and the overcoming of Muller's ratchet. A full analysis would however need to take into account changing population sizes, organism sizes, niche sizes, and mutation rates that may be associated with a change in the generation time.

Since the interests of the mitochondrial genome are tightly tied to the interests of the organisms within which they reside, if the organism benefits from a shorter generation time, so will the mitochondrial genomes.

Any talk of the mitochondrial genome sabotaging the existence of the organism so as to force it to engage in sex has to rely on kin selection. The benefits to closely related kin of the mitochondrial genomes that engage in sabotage have to outweigh the loss of mitochondrial genomes as a result

of this sabotage. The kin are the other mitochondrial genomes of the species. And the benefit to the kin is the removal of nuclear alleles from the gene pool that conferred a resistance to sex. In so doing the mitochondrial genomes maintain a potentially large ability to adapt to a changing external environment as a result of nuclear genetic recombination. Mitochondrial genomes that don't enforce sex to survive upon the nuclear genomes will find the nuclear genomes engaging in sex less and less frequently, leading to a worsening ability to adapt and ultimately the loss of the species.

To understand how kin selection of mitochondrial genomes works, consider a population of organisms with the optimal fitness producing lifespan for the population, except for a single organism with an allele A coding for a lifespan y years longer than optimal for the population. Because y is larger than normal, the organism with allele A has increased fitness and will bear more offspring. A longer generation time may increase the fitness for the organism, but reduces the mean fitness for a population. Because of the increased organismal fitness the population of allele A will eventually become fixed, at which point each successive generation will experience a log fitness loss of, say, c. τ generations after this the total aggregated log fitness loss will be in excess of $c\tau$. A value that will grow without bound as τ increases. But this entire fitness loss could have been avoided at small cost if a single mitochondrial genome had simply killed the original organism. Thus by kin selection there is an advantage for the mitochondria to kill organisms that would have survived longer than the optimal lifespan.

In summary, nuclear alleles strive for near immortality at a cost to the species, while the mitochondrial genome supports frequent mortality. Given mortality the nuclear genes will bend to the interests of the mitochondrial genome, and support sex as a means of resetting mortality.

3.1 Mortality and metabolism

So far the mitochondrial genome has been viewed as imposing a time limit on the life of the organism. But mitochondrial genomes aren't armed with a stopwatch, and even if they were this might not be the most appropriate way of doing things. Instead it seems more likely that lifespan might be tied to something easier to measure such as the aggregate inputs or outputs of the mitochondria. This might explain the observation that lifetime energy consumption per unit of body weight is roughly constant for related species[55]. This might also explain the correlation between obesity (the result of greater metabolic inputs), and shortened lifespan. The world beyond the organism might be viewed as containing energy, and the evolutionary mandate is to gather up enough energy to create another organism as quickly as possible.

3.2 Mortality and species

Life exists in a dynamic equilibrium. New species are constantly being formed. Meanwhile existing species are evolving. Some evolve to live longer, and then they go extinct through a failure to adapt in comparison to other species that live a shorter length of time.

Successful organisms might come from a long line of successful organisms, but successful species usually come from a long line of failures; failures to live longer that is.

There is almost no limit to the length of time the nuclear genome would like the organism to live, since the longer the organism lives the more offspring are possible. There is however a species specific length of time the mitochondrial genome would like the organism to live. This length of time relates to how quickly the organism can produce successful offspring, as well as the fitness loss to the mitochondrial genome that comes with a longer lifespan.

If species faced little competition, then nuclear mutations that increase lifespan would occur, extending species lifespans. On the other hand, the more intense the competition between species, the closer to the mitochondrial genome's preferred length of time to live that species should be found. Anecdotally, consider the very long lifespan of the Galápagos tortoise, which probably faces little interspecies competition. Similarly, the naked mole-rat occupies a relatively unique ecological niche, subterranean burrows in the Horn of Africa that often have little oxygen, and it exhibits a very long lifespan for its size[56]. And finally, the bristlecone pine, *Pinus longaeva*, generally grows in harsh environments where most other plants are unable to grow, and has the longest lifespan of all known non-clonal organisms[57].

Some species are hypothesized to go extinct due to an increased lifespan and consequent failure to adapt. For this hypothesis to be valid it is desirable that proteins evolve more slowly than the rate at which new species come into existence. Otherwise most new species might evolve proteins to overcome the constraints on lifespan and go extinct. Proteins evolve slowly. Comparing 2,820 orthologous humans and mice genes, a median non-synonymous substitution rate per site per year of 3.9×10^{-10} has been reported[58]. If we very conservatively require 5% non-synonymous sequence divergence to effect a new lifespan affecting function, a 5% non-synonymous sequence divergence occurs every 130 million years. Even the fastest evolving gene pair in that study only evolved at a rate of 3.81×10^{-9} substitutions per site per year, or 5% every 13 million years. On the other hand, new species are formed relatively quickly. For instance there are at least 495 known species of Muridae that are estimated to have evolved in the last 22 million years[59]. Assuming an equally balanced tree, this amounts to a species diverging into two species after every 2.5 million years. Thus the nuclear genome appears unable to out-evolve the rate of species creation.

The larger the species population, the more rapidly the nuclear genome might be able to mutate to overcome its mitochondrial genome derived constraints and extend the species generation time. This would reduce the mean fitness of the population relative to other species, and so reduce the population. In this way populations may be kept in balance.

Viewing species from a mitochondrial perspective provides new insight into the question of whether species can be considered as a unit of selection above and beyond selection acting on the individual organisms and nuclear alleles that make up a species[60]. This seems more likely if traits such as lifespan are coded for by the mitochondrial genome of the species.

4 Aging

So far we have seen how the mitochondrial genome would benefit from enforcing mortality. It would lead to a shorter time between successive generations, and this would lead to an increased ability to adapt. But he idea that the mitochondrial genome is actually causing mortality may seem like a bridge too far. It will thus be necessary to examine the causes of mortality, and especially

aging, to see if the mitochondrial genome is implicated. In particular, we will explore the roles of mitochondrial ROS production in causing apoptosis, cellular senescence, and age-related diseases.

Aging is a process of declining ability to respond to stress over time, and an increase in the probability of death. Cellular senescence is a key mechanism of aging. As previously mentioned mitochondria appear to play key roles in both apoptosis and in cellular senescence.

Almost all eukaryotic organisms appear to age, while under suitable conditions symmetrically dividing prokaryotic populations must be immortal[61, 62]. It seems reasonable here to hypothesize that the primary purpose of aging is to cause organism death as a means of promoting frequent sex. It is also reasonable to hypothesize that the aging phenotype is the result of the complex back and forth between mitochondrial and nuclear pro-aging genetic elements, and nuclear genome anti-aging genetic elements. This back and forth occurs on a stage in which the most successful nuclear genome anti-aging adaptations die out. The development of brand new mitochondrial genome pro-aging genetic elements must be a very rare event because the effective size of the mitochondrial genome is very small.

Hypothesis 4: Aging is a process in which the mitochondrial genome enforces mortality.

Some support for this hypothesis is given by age getting reset by the mitochondrially desired process of sex, rather than in response to some other event. Further evidence will be provided in sections 4.3 and 4.4 that examine aging in unicellular and multicellular organisms.

4.1 The duality hypothesis

Along with mitochondrial genome derived genetic elements that cause aging, it is possible to also have nuclear genes that cause a shorter lifespan or aging. If such nuclear genes only caused aging they would be selected against, but if such genes also played some separate and important life giving role, they need not be. We should thus expect pro-aging nuclear genes to be pleiotropic; also exhibiting some beneficial function from a nuclear genome perspective. To be precise, the gain in organism fitness obtained over a shorter lifespan should exceed the loss in organism fitness associated with that shorter lifespan.

Hypothesis 5: Pro-aging nuclear genes will also exhibit some vital life-enhancing function.

The duality hypothesis suggests there will be some difficulty in properly determining the pro-aging pathways. Not only is there the effect of gene duplication to contend with, but each gene can be expected to have both life giving and lifespan reducing functions.

The duality hypothesis appears capable of explaining the otherwise extraordinary observation that most of the apoptotic death effectors also play some vital, non-death related function in the cell[63]. For unicellular organisms this is straightforward. For multicellular organisms there needs to be a link between cellular apoptosis and organismal death, such as a link from cellular apoptosis to thymic involution and hence increased disease susceptibility. This is explored in subsection 4.4.2.

An immediate corollary to the duality hypothesis is given below.

> Corollary 5.1: Anti-aging interventions can commonly be expected to exhibit reduced biological fitness in the evolutionary environment.

The corollary to the duality hypothesis suggests that interventions intended to extend lifespan will have to tread carefully so as to not interfere with any life-enhancing role.

One area where the corollary to the duality hypothesis is less likely to be problematic is anti-aging interventions that affect energy uptake. The evolutionary environment was punctuated by periods of feast and famine. Consequently the organism was evolutionarily programmed to take up large amounts of energy when times were good, so that that energy was available when times were bad. Artificially reducing energy uptake despite times being good might fool the organism into believing it is a low energy environment, and thus needs to be given longer to complete its biological program.

A second area where the corollary to the duality hypothesis is less likely to be problematic is anti-aging interventions that target pathways for which the vital life-enhancing function occurs during development.

4.2 Reactive oxygen species

The mitochondrion is a major source of reactive oxygen species (ROS). Complexes I and III of the electron transport chain both leak superoxide ($O_2^{\bullet-}$), with roughly 0.2-2.0% of all oxygen consumed by the mitochondria ending up as $O_2^{\bullet-}$[64]. Complex I leaks towards the mitochondrial matrix, while complex III leaks towards both the matrix and the intermembrane space[64]. $O_2^{\bullet-}$ gets converted into the more stable ROS hydrogen peroxide (H_2O_2) by superoxide dismutase. H_2O_2 is stable by itself, but in the presence of Fe^{2+} it undergoes the Fenton reaction, producing an extremely reactive hydroxyl radical ($HO^{\bullet}$), a hydroxide ion (OH^-), and Fe^{3+}[65]. As the name suggests, ROS are highly reactive, and unless neutralized by antioxidants, can cause damage to the nucleic acids, proteins, and lipids that make up the cell[66]. Cellular membranes are weakly permeable to H_2O_2, but not $O_2^{\bullet-}$[67, 68, 69].

In mammals mitochondrial ROS production is known to increase with age[70].

By physically damaging the components of the cell, ROS may have been the original mechanism through which the mitochondrial genome caused the cell to age and die. The nuclear genome might be expected to develop proteins to oppose such aging and death, while, within limits, the mitochondrial genome will seek to promote it. Consequently, there has been much evolutionary plastering over of ROS as a mechanism of aging, but the observed result is something that has been done with a light hand. Too heavy of a plastering over and the species goes extinct. The mito-nuclear back and forth and the need that things be done with a light hand may explain the seeming complexity of the apoptotic pathway which probably involves the concerted effect of close to 100 proteins.

Hypothesis 6: ROS production is the fundamental mechanism by which the mitochondrial genome causes aging.

The evidence for ROS production as the mitochondrial mortality mechanism is reviewed when we consider aging in unicellular and multicellular organisms in sections 4.3 and 4.4.

Superoxide dismutase (SOD) converts $O_2^{\bullet-}$ into O_2 and H_2O_2. Eukaryotes contain several forms of SOD. SOD1, a Cu-Zn-SOD, is found in the cytosol. SOD2, a Mn-SOD, is found in the mitochondrial matrix, and SOD3, another Cu-Zn-SOD, is found extracellularly in mammals and most chordates.

Peroxidases break down H_2O_2 to water and oxygen. Three common peroxidases are peroxiredoxins (Prxs), glutathione peroxidase (GPx), and catalase. Prxs exist in the cytosol, mitochondria, and peroxisomes. GPx is found in the cytosol and mitochondria. Catalase has an extremely high turnover rate. In most eukaryotes catalase is only found in peroxisomes, and not in the cytosol. The Ctt-1 gene of *Saccharomyces cerevisiae* and the ctl-1 gene of *Caenorhabditis elegans* are two exceptions. The frequent lack of a cytosolic catalase may be the result of H_2O_2 being used to signal species lifespan.

Seemingly opposing the perspective that ROS cause the cell to age, mice with an error-prone version of the mtDNA polymerase γ display an aged phenotype without an increase of ROS in embryonic fibroblast cells[71]. It is as if the mtDNA mutations alone are directly responsible for the aged phenotype, but the natural mtDNA mutation rate appears far too small to have a significant effect[72]. Looking at various tissues it was subsequently shown that mutator mice do show slightly elevated H_2O_2 as they age[73]. It was also shown that age-dependent cardiomyopathy in mutator mice could be attenuated by mitochondrially targeted catalase[74]. The evidence from mutator mice is sufficient to cast serious doubt on the theory that ROS induces more ROS damage creating a vicious cycle, but still leaves open a role for ROS as a residual signaling-like mechanism in aging.

Consistent with ROS being the evolutionary mechanism through which the mitochondria controls lifespan, comparisons between different species have generally shown a negative correlation between ROS levels and lifespan[75, 76].

Within individual species the overexpression of antioxidant enzymes is generally associated with an increase in lifespan[77][75][Table 4]. Similarly the deletion of genes coding for antioxidant enzymes generally results in a decrease in lifespan[75][Table 5]. Exposure to antioxidants compounds also often increases lifespan[75][Table 6]. These effects are however by no means universal. The reason for this lack of universality may be because ROS are also used by the cell as signaling molecules and for the killing of bacteria[78, 79]. Contradicting the theory being developed, mild exposure to ROS generating compounds can increase lifespan[75][Table 7]. Similarly mutations that increase ROS production can sometimes increase lifespan[75][Table 8]. This is known as hormesis.

In humans, mitochondrial DNA haplogroup D4a is correlated with extreme longevity[80]. One of four mutations associated with this haplogroup is at position 14,979[80]. This places it in cytochrome b, a core member of complex III. Thus it is possible that a reduction in cytosolic ROS from complex III could be the cause of this extreme longevity.

ROS can be expected to be produced in proportion to the extent to which the mitochondrion is used

to produce ATP. If this ROS proves harmful to the cell, this could explain why caloric restriction is capable of extending an organism's lifespan[81]. Caloric restriction will result in less energy being available to the cell, and thus a lower rate of ATP and ROS production by the mitochondrion.

4.3 Aging in unicellular organisms

The yeast *Saccharomyces cerevisiae* is a unicellular organism that can exist as either a haploid or a diploid[82]. In yeast cell division is asymmetric with mitosis involving a small daughter cell budding off from a larger mother cell[82]. Haploid lab yeast strains are often modified to prevent mating type switching[83]. This may have little relevance for most lab studies, but may be significant here as we are interested in the behavior of yeast in its evolutionary environment.

Two different measures of lifespan are used in yeast. Replicative lifespan (RLS) is the number of daughter cells a mother cell can produce[84]. Chronological lifespan (CLS) is the length of time a cell remains viable after reaching stationary phase and cell cycle arrest in nutrient deprived media[84]. RLS is possibly more relevant here. Surprisingly, but consistent with the model developed here, the RLS is finite, and is typically somewhere around 25 cell divisions[85]. Chronologically aged yeast appear to undergo a form of programmed cell death similar to the apoptosis of multicellular organisms[86].

Deletion or overexpression of SOD in yeast increases lifespan in a manner largely consistent with the idea that $O_2^{\bullet-}$ rather than H_2O_2 causes aging in yeast. Deletion of the gene for cytosolic SOD1 dramatically reduces RLS[87, 88]. Overexpression of SOD1 (in conjunction with cytosolic catalase) has no effect on RLS[89]. Deletion of the gene for SOD1 significantly reduces CLS[90, 88]. Overexpression of SOD1 increases CLS[91]. Deletion of the gene for SOD2 decreases or has no effect on RLS[87, 88]. Deletion of the gene for SOD2 significantly reduces CLS[90, 88]. Overexpression of SOD2 increases CLS[91].

A major paradigm in yeast aging research has been studying the effect of calorie restriction (CR), which typically involves growing yeast on a 0.5% or lower, rather than a 2%, glucose medium[92]. There is some uncertainty, but CR does not appear to have a major impact on RLS in yeast[93]. On the other hand, CR extends CLS[92]. A proposed mechanism involves CR causing increased mitochondrial respiration; as opposed to fermentation[94]. This will increases the production of $O_2^{\bullet-}$, which is converted to H_2O_2 by the action of SOD. An increased concentration of H_2O_2 activates the cell's antioxidant defense system which includes production of SOD1, SOD2, and cytosolic catalase[95, 96]. SOD1 and SOD2 will then presumably somewhat paradoxically reduce the concentration of $O_2^{\bullet-}$. How a reduction in $O_2^{\bullet-}$ leads to an increase in CLS doesn't appear to have been determined.

In yeast, two mechanisms of resetting RLS based age are known. The first is mitosis, in which just the daughter bud's replication based age appears to get reset[97]. The second is meiosis[98]. The resetting of RLS based age by meiosis is dependent on the mid-meiosis transcription factor Ndt80 which is expressed after DNA replication and Holliday junction formation, and activates around 200 genes[98, 99, 100]. Mutants deficient in Ndt80, or Cdc5 which it regulates, show reduced Holliday junction joint molecule resolution, and reduced crossing over[101]. Importantly, transient induction of Ndt80 restores replicatively aged cells to a young state, and a nucleolar morphology to that of a replicatively young cell[98].

Failure of haploid yeast cells to mate in the presence of the opposing mating pheromone leads to apoptosis[102]. This may represent a more direct form of mortality in the absence of meiosis than aging based on the number of cell divisions. It would allow yeast cells to survive for extended periods when no mating partner exists, but as soon as mating partners exist they are expected to mate.

Thus in yeast we see several of the core predictions of HEPM: aging and mortality in the absence of sex, apparently intentional mortality, and the induction of youth being closely tied to recombination. What goes against HEPM is replicative youth in mitotic daughter cells.

4.4 Aging in multicellular organisms

In the wild, most animals exhibit organismal senescence, displaying increased mortality rates at increased chronological ages[103]. The existence of organismal senescence can be readily explained by HEPM.

Aging in multicellular organisms differs from yeast. Some of the principles are likely the same: mitochondrial ROS leading to organismal death as a means of promoting sex. But there are important differences relating to multicellularity. In particular, rather than committing apoptosis, cells that wish for the organism to die need a mechanism to reach consensus before they try to kill the host organism.

This subsection might apply more broadly, but will primarily be focused on the mechanisms of aging in vertebrates.

4.4.1 Cellular senescence

Senescent cells fail to divide, resist apoptosis, and usually exhibit the senescence-associated secretory phenotype (SASP)[104]. The SASP is frequently pro-inflammatory, proapoptotic, and is capable of inducing senescence in both nearby and distant non-senescent cells[104, 105]. Natural killer cells are often capable of clearing senescent cells[106]. However, with age, the number of senescent cells is found to accumulate, and this is implicated in various age-related diseases[107]. The SASP appears to be caused by a pathway that is triggered by mitochondrial ROS[108, 109]. A reduction in mitochondrial content is known to prevent senescence and attenuate the SASP[110]. Since the SASP is implicated in various age-related diseases, and senescent cells can induce senescence in other cells while themselves being resistant to apoptosis, the SASP represents an ideal mechanism for mitochondria to ultimately cause organismal death.

The threshold theory of cellular senescence postulates that once the senescent cell burden exceeds some threshold level, the induction of senescence outpaces the rate of clearance, and senescence spreads through the organism due to the paracrine and endocrine activity of the SASP[106]. The threshold theory dovetails nicely with the idea that senescent cells need to reach a consensus or quorum before attempting to cause organismal death.

Consistent with the duality hypothesis cellular senescence plays important life-giving roles during organismal development and wound healing[111, 112].

Moderate doses of certain antioxidants are known to inhibit cellular senescence[113]. Disruption of the electron transport chain is known to promote senescence[114]. This is consistent with mitochondrial ROS leading to senescence.

4.4.2 Immunosenescence

Immunosenescence is the gradual decline in the efficacy of the immune system with age[115]. Multiple factors contribute to immunosenescence[116]. A major factor is the effect of thymic involution[115]. The thymus is the site of T-cell maturation. Thymic involution is the gradual shrinking of the thymus with age. Thymic involution appears to include an increased thymocyte apoptosis and reduced thymocyte proliferation in the aged thymus[117]. This leads to a reduction in naive T-cell output that likely contributes to immunosenescence[118]. Thymic involution in adults and adolescents, but not in infants and children, may be caused by thymic epithelial cells increasingly exhibiting SASP[119]. ROS are also implicated as a cause of thymic involution[120]. Thymic involution is common to nearly all organisms possessing a thymus[121], although the selective pressures for thymic involution appear not well understood. The possibility that thymic involution is intended to cause organism death and therefore promote early genetic recombination doesn't appear to have been considered.

4.4.3 Age-related diseases

Many age-related diseases involve senescence or the SASP:

- Cardiovascular disease. Myocardial infarction (heart attack) and stroke are both the result of atherosclerosis. Age is an independent risk factor for the development of atherosclerosis and premature biological aging such as in patients with Werner syndrome or Hutchinson Gilford progeria syndrome accelerates the development of atherosclerosis[122]. The SASP is implicated in atherosclerosis[123].
- Cancer. Age is a primary risk factor for most cancers. One model of tumorigenesis holds that the immune system is capable of resolving many cancers in the young, but that immunosenescence leads to reduced ability to do so in the elderly[124, 125]. Oncogene-induced senescence is widely considered a tumor suppressor. However the SASP can also promote tumorigenesis[126]. In addition senescent cells may be able to escape oncogene-induced senescence leading to tumor progression[127]. Perhaps senescence in the context of a premalignant lesion should be viewed as a decision by the cells making up the lesion to leave it to the immune system to decide upon the organism's fate.
- Alzheimer's disease. Alzheimer's disease is of the elderly that results in neuronal apoptosis. Allele 4 of apolipoprotein E (ApoE) is a major risk factor for Alzheimer's disease[128]. It might thus be expected to be selected against. However, like the apoptotic death effectors that also have a non-death related function, ApoE4 is correlated with a higher level progesterone in women, increasing the chances of conception and successful pregnancy[129]. From the perspective of the nuclear genome this represents a case of antagonistic pleiotropy, but from a mitochondrial genome perspective it seems purely adaptive. SASP astrocytes may play a role in Alzheimer's disease[130].

- Diabetes. Insulin promotes the cellular absorption of glucose. Type 2 diabetes involves a combination of inadequate insulin production by β-cells in the pancreas and cellular insulin resistance. The production of insulin by β-cells appears to be limited in type 2 diabetes because many of the β-cells have committed apoptosis[131]. Insulin resistance is a reduced ability to absorb insulin and use it to take up glucose. Senolytics are drugs that kill senescent cells. Senolytic drugs are known to be able to prevent and alleviate insulin resistance in mice[132].
- Infectious diseases. Increased susceptibility and death due to infectious diseases with age seems likely to be the result of immunosenescence including thymic involution.

The picture that emerges is of many age-related diseases having cellular senescence as a common mechanism, and these different age-related diseases merely being different tissue or organ specific expressions of cellular senescence.

Hypothesis 7: Cellular senescence is a key downstream mechanism of aging in vertebrates.

Section 4.5 dealing with the molecular pathways of cellular senescence will show cellular senescence exists downstream from mitochondrial ROS production.

Aging in vertebrates conforms to HEPM: mortality rates that increase with age creating an upper bound on lifespan, and mortality reset by sex. Moreover, mitochondrial ROS appears to cause the SASP, which then causes various age-related diseases.

4.4.4 Apparent intentionality of variation in lifespan across species

Mice have a gestation time of slightly less than 1 month, reach sexual maturity in about 2 months, and have a reproductive lifespan of 7 to 8 months. Thus a rough estimate of the mean generation time is 0.5 years. The mean generation time for humans in their evolutionary environment is around 30 years[133], or 60 times longer than mice.

The biology of humans and mice is very similar; they diverged 87 million years ago[59]. A 60 fold difference in mean generation times thus suggests mean generation time may be under genetic control.

Mice display many of the same causes of death as humans[134]. This seems remarkable given they have a much shorter lifespan. Cancer for instance is the leading cause of death in mice and ranks second for humans[134]. That 1 year old mice frequently die from cancer while 1 year old humans rarely do strongly suggests that death from cancer is an evolutionary adaptation. This seems even more likely when consideration is given to the relative sizes and numbers of cells in mice and humans. 1 year old mice are much smaller, yet they die from cancer more frequently. The alternative hypothesis is that mortality is a maladaptation but that closely related humans have found a way to largely beat cancer and all the other causes of death that 1 year old mice experience. This alternative hypothesis seems implausible. Thus mortality appears to be adaptive.

Mice don't die at random. Presumably, the older the mouse, the more likely it is to die. In humans this phenomenon has been codified by the Gompertz-Makeham law[135]. The death rate at age y, is given by,

$$\alpha e^{\beta y} + \lambda$$

for constants α, β, and λ. Based on this form, for the 2019 U.S. Social Security area population, the mortality rate doubles every 8 or 9 years. This means the probability of death throughout the reproductive lifespan is small, but it becomes substantial some time thereafter. Thus sexuality seems to have evolved to ensure the vast majority of organisms are capable of bearing offspring before they die. This is consistent with the hypothesis that the purpose of mortality is to force the nuclear genome to engage in sex.

4.4.5 Periodontitis

Severe chronic periodontitis affects about 27% of the global population over the age of 40[136]. Periodontitis appears to be an independent risk factor for cardiovascular disease, cerebrovascular diseases, certain cancers, diabetes, and rheumatoid arthritis[137]. In one study the relative risk of all cause mortality for individuals with periodontitis compared to no periodontal disease was 1.46[138]. This was after adjusting for other factors that may influence the outcome: age, sex, race, education, poverty index, marital state, systolic blood pressure, total cholesterol concentration, diabetes, body mass index, physical activity, alcohol consumption, and cigarette smoking.

The presence of senescence cells in periodontal tissue might explain how periodontitis is capable of affecting all cause mortality to such a significant extent[139, 140]. A hypothesis might be that bacteria activate the immune system, leading neutrophils to release ROS to destroy the bacteria, but ROS are also harmful to periodontal tissue, causing telomeric DNA damage that leads to senescence[139]. Assuming components of the SASP are able to spread in an endocrine-like fashion, the SASP could then influence the health of more distant cells.

4.5 Molecular pathways of cellular senescence in vertebrates

Cellular senescence comes in different flavors[141]. Replicative senescence limits the number of divisions a cell can make and is linked to mitotic telomere shortening. Oncogene-induced senescence is in response to DNA damage. Stress-induced senescence is the induction of senescence in response to chemicals such as H_2O_2. All three result in growth arrest, the SASP, and morphological changes. We are most interested in stress-induced senescence, as it most closely reflects the action of mitochondrial ROS.

One plausible method of aging is for there to be an intracellular timekeeper that records the age of the cell, and organismal age is then a function of the age of the cells that make it up. But what fundamentally determines the age of the cell? ROS levels appear to reflect the rate of passage of time as the cell ages, but ROS levels do not reflect the age of the cell. One possibility would be for age to be determined based upon the levels of some other chemical that gradually builds up

or is depleted over time. This has the problem that concentrations of most chemicals are likely to undergo stochastic fluctuations, and be too variable to stably convey an accurate age signal over a period of years. There is however one chemical whose concentration is invariable over the life of the cell: DNA. If cellular age was encoded on DNA it could form a stable signal. This is what appears to happen. Telomeres undergo occasional irreversible ROS induced damage, leading to telomere shortening, and this appears to play the role of the intracellular timekeeper.

A proposed molecular pathway leading from mitochondrial $O_2^{\bullet-}$ production to senescence is shown in Figure 2 and expanded upon below. The molecular biology of senescence is still being elucidated, and other plausible pathways exist. Gene duplication, and the fact that every nuclear pro-senescence gene can be expected to have both pro-aging and vital life-enhancing effects (the duality hypothesis), creates some difficulty in determining the relevant pathways with certainty.

4.5.1 ROS

As shown at the top of Figure 2, it is proposed that mitochondrially produced $O_2^{\bullet-}$ gets converted into the stable ROS H_2O_2 by SOD. H_2O_2 is weakly membrane permeable and should over the course of minutes to hours be capable of migrating to the nucleus[67, 68]. The Fenton reaction then produces the highly reactive $HO^{\bullet}$ from H_2O_2.

The Fenton reaction involves the oxidation of Fe^{2+}. In humans genome wide association studies have found the heme metabolism pathway is related to lifespan, and that serum iron has been found to correlate negatively with lifespan[142]. Generally speaking, mild iron deficiency and iron chelators have been found to increase lifespan in various species, while excess iron has been found to promote aging[143]. This is understandable if increased iron leads to increases in the production of $HO^{\bullet}$. In *S. cerevisiae* the effects of iron appear mixed. Increased levels of iron brought about by deletion of the inositol phosphosphingolipid phospholipase C gene (isc1) showed increased H_2O_2 sensitivity, a shorter chronological lifespan, and increased evidence of apoptosis[144]. Opposing this, iron supplementation, again in *S. cerevisiae*, has been reported to extend chronological lifespan[145]. The relevance of this mixed evidence is muted by the lack of clarity on how ROS levels modulate lifespan in unicellular organisms.

Interestingly, the Fenton reaction is known to be greatly enhanced in the presence of the DNA sequences AGGG and GGGG[146]. These sequences form part of the telomeric repeat for a majority of multicellular organisms, with TTAGGG being the canonical sequence for opisthokonts; which includes animals and fungi[147].

Note that the presence of H_2O_2, as opposed to $O_2^{\bullet-}$, as a part of the proposed pathway to senescence is slightly uncertain. This is because microsomes, peroxisomes, and other cytosolic enzymes are also capable of producing substantial amounts of H_2O_2[148].

4.5.2 Telomeric damage

As further shown in Figure 2, $HO^{\bullet}$ is capable of producing a range of DNA damage, including frequently converting guanine, G, into 8-oxoguanine (8-oxo-G)[149]. 8-oxo-G is detected and removed by the base excision repair (BER) machinery. In BER, 8-oxoguanine glycosylase (OGG1)

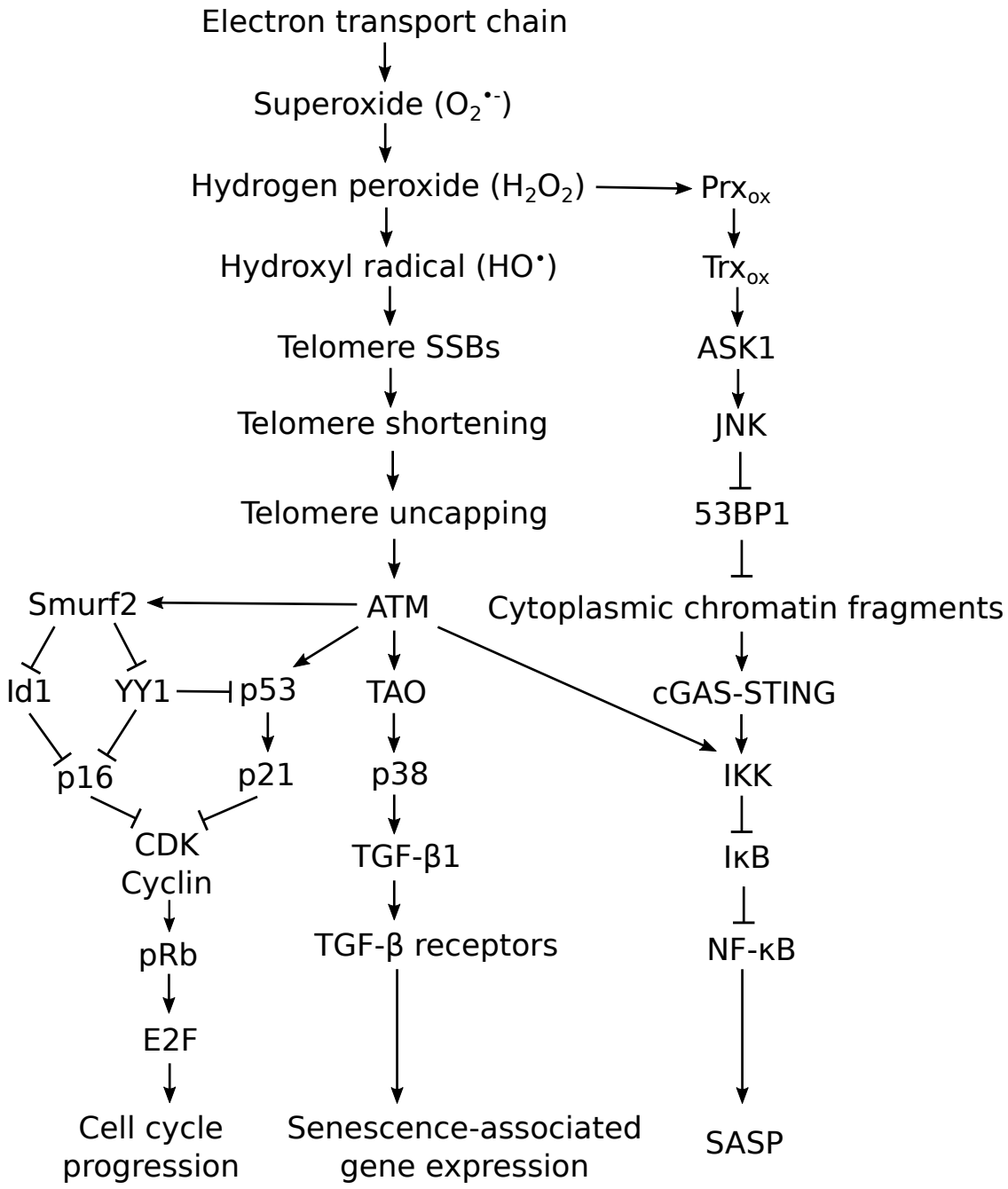

Figure 2: A proposed molecular pathway leading from mitochondrial superoxide production to senescence in vertebrates and possibly other species.

removes 8-oxo-G and creates a single strand break (SSB) in the DNA backbone, which is normally immediately filled with the correct base and ligated[150]. In telomeres the SSB repair steps appear impaired[151]. This may be due to the action of telomeric repeat-binding factor 2 (TRF2) which associates with the telomeres[152]. Thus $HO^{\bullet}$ is capable of producing longer lasting SSBs.

Unrepaired telomeric SSBs will lead to telomere shortening when the cell next divides[153]. In non-proliferating cells, two unrepaired SSBs within approximately 1 or 2 turns of the DNA double helix (10 to 20 base pairs) located on opposing strands are likely to lead to a double strand break (DSB)[154, 155], creating telomere shortening.

The DNA damage response (DDR) might view chromosome ends as DSBs and attempt to randomly repair them by joining chromosomes together[156]. TRF2 binds to telomeres and usually prevents the induction of the DDR at chromosome ends[156]. If telomeres shorten sufficiently they become uncapped, adopting a linear conformation, in which the remaining TRF2 appears sufficient to prevent end joining, but insufficient to prevent DDR signaling by ataxia telangiectasia mutated (ATM)[157], leading to persistent ATM DDR signaling by the telomere.

The occurrence of multiple persistent DDR signals from multiple telomeres is sufficient to induce cellular senescence[158].

Support for persistent ATM DDR signaling by telomeres as the indicator of age for the cell is provided by a number of observations. Telomeric damage irreparably appears to be evolutionarily conserved; it occurs in both yeasts and humans[159, 160]. Live-cell imaging experiments show all persistent DNA damage foci to be associated with telomeres[161]. There is an age-dependent increase in the number of telomere-associated foci that occurs irrespective of telomere length[161]. Shortened telomeres are associated with aging, as well as mortality risk[162]. Intracellular ROS levels are known to accelerate telomere shortening in an exponential manner[163]. SOD3 is known to reduce the rate of telomere shortening[164]. And all eukaryotes appear to have linear chromosomes with telomeres rather than circular chromosomes or circular genomes like bacteria and archaea.

Consistent with the duality hypothesis, the ATM mediated DDR provides both a pro-aging signal, and by signaling for the repair of DSBs, a fitness enhancing function.

4.5.3 ATM

The DSB DDR in the form of persistently phosphorylated ATM appears to be at the hub of the senescent phenotype. Activated ATM appears to be responsible for cell cycle arrest, the expression of a number of genes associated with senescence, and the SASP. This is enumerated in the following sections.

Arguing for the model of activated ATM as the cause of senescence, elevated levels of activated ATM have been found with age in naturally aged and acceleratedly aged mice. and reducing ATM activity has been found to reduce senescence[165]. Similarly inhibition of ATM has been found to ameliorate senescence[166]. In this latter result, ATM was hypothesized to phosphorylate a component of an ATPase responsible for acidification of the lysosome leading to lysosomal dysfunction. Seemingly contradicting these findings, decreased ATM levels along with reduced p53 activity have been found in older mice[167]. Similarly, declining levels of ATM have been reported with replicative

passage, knocking down ATM has been reported to accelerate senescence, and activation of ATM has been reported as being capable of clearing replicative senescence[168]. Part of the reason for the seeming discrepancy in these results may be due to the difference between ATM expression levels and phosphorylated and activated ATM, and the study of replicatively induced as opposed to DNA-damage-induced or stress-induced senescence.

4.5.4 p16, p53, p21, and cell cycle arrest

As shown in the leftmost fork of Figure 2, activated ATM is able to phosphorylate and activate Smurf2[169]. Smurf2 is a ubiquitin ligase, and its targets include the transcriptional repressors inhibitor of DNA binding 1 (Id1) and yin yang 1 (YY1)[170, 171]. Id1 and YY1 repress the transcription of cyclin-dependent kinase inhibitor p16[172]. The pathway from ATM's activation to activation of p16 doesn't appear to be well studied, and it is possible other pathways exist different from this one.

Supporting a role for p16, p16 increases with age, and has even been proposed as a biomarker of aging[173, 174]. p16 expression is also significantly elevated in senescent cells[175].

p16 binds specifically to cyclin dependent kinases (CDKs) 4 and 6 preventing them from phosphorylating retinoblastoma protein (pRb)[172]. In its phosphorylated form pRb would have changed conformational form releasing bound E2F transcription factors[172]. The E2F transcription factors are responsible for the transcription of the genes necessary for the G1 to S phase transition, or in the event of prolonged E2F expression, apoptosis[172, 172].

Activated ATM is also able to phosphorylate and stabilize p53, a key regulator of cell fate. p53 positively regulates transcription of the cyclin-dependent kinase inhibitor p21[176]. p21 binds to and non-specifically blocks the activity of CDKs again preventing the G1 to S phase transition[177].

In addition, YY1 acts as a negative regulator of p53[178].

Thus, activated ATM is able to arrest the cell cycle through multiple means.

4.5.5 p38 and senescence-associated gene expression

As shown in the central fork of Figure 2, in addition to arresting the cell cycle, ATM is also capable of phosphorylating and activating thousand and one amino acid (TAO) kinases[179]. TAO kinases are MAPK kinase kinases (MAP3K), which activate MAPK kinases (MAP2K) kinases, which activate p38 MAPK[180]. Activated p38 is known to both mediate apoptosis and in specific circumstances cell survival[181]. Activated p38 is also known to cause overexpression of transforming growth factor-β1 (TGF-β1)[182]. Osteonectin, apolipoprotein J, and fibronectin are commonly over-expressed in senescence[183]. TGF-β1 appears to cause an increased expression of mRNA for these three genes, as well for its own receptor[184]. This increased expression is eliminated by antibody neutralization of TGF-β1 or its receptor. Thus activated ATM may be capable of producing part of the phenotype associated with senescence.

4.5.6 Retrograde signaling, NF-kB, and the SASP

Finally, as shown in the rightmost part of Figure 2, the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is capable of being activated through two mechanisms. NF-κB appears responsible for the SASP[185].

The first mechanism of activating NF-κB is by cytosolic ATM[165].

The second mechanism appears to only occur in senescent cells and involves retrograde signaling from the mitochondria to the nucleus. Peroxiredoxin (Prx) reduces H_2O_2 to H_2O, and in the process becomes oxidized. Prx is reduced back to its active form by the action of thioredoxin (Trx), which itself becomes oxidized. Oxidized Trx is hypothesized to act as a H_2O_2 sensor[186]. Reduced Trx forms an inactive complex with apoptosis signal-regulating kinase 1 (ASK1), a MAP3K. Oxidation of Trx produces a conformational change which leads to the activation of ASK1[187]. ASK1 activates c-Jun N-terminal kinase (JNK)[188]. JNK interacts with p53-binding protein 1 (53BP1) through mechanisms that are still being elucidated[189]. 53BP1 plays a role in the non-homologous end-joining DSB repair pathway. The absence of 53BP1 leads to the formation of cytoplasmic chromatin fragments (CCF)[109]. The presence of double stranded DNA in the cytosol triggers the cyclic GMP-AMP synthase (cGAS) – stimulator of interferon genes (STING) pathway[190]. STING activates the IκB kinase (IKK), which then phosphorylates IκB leading to IκB degradation via the ubiquitin-proteasome pathway, freeing NF-κB from its association with IκB, and allowing NF-κB to enter the nucleus[191].

Taken together these pathway show a route leading from mitochondrial ROS production to cellular senescence. This provides evidence for the claim that the mitochondrial genome seeks to enforce mortality, and in so doing maximizes the fitness of the environment within which it is embedded.

5 Addressing age-related diseases

Today there exist many one-disease-at-a-time approaches for addressing age-related diseases. These approaches are likely to only be weakly effective. The elimination of all forms of cancer for instance is only expected to extend lifespan in the U.S. by 3 years[192]. If one age-related disease doesn't kill you, another one will.

Proposed multi-disease approaches for addressing age-related diseases are split across the nine different hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication[162]. There isn't a clear consensus on the relationship between the different hallmarks of aging, and what causes what.

Age-related diseases and their mechanisms may be divided into three classes. Those that exist downstream of mitochondrial ROS production; these may be considered fundamental and of mitochondrial origin. Those that exist due to an evolutionary trade-off between the nuclear genome's desires for immortality and reproduction; these are probably rare, and may also be considered fundamental, but of nuclear origin. And those that exist merely because they occurred infrequently enough in the evolutionary environment to be selected against; these may be considered as ancil-

lary. These ancillary diseases may exert a significant toll if the optimal lifespan for the species is increasing, or if most of the fundamental diseases have been cured.

If the fundamental mitochondrial origin age-related diseases were eliminated, and mortality rates dropped to match those of a U.S. 20 year old in 2019, the lifespan of men would increase to 927 years, and for women it would increase to $2,469$ years. These lifespans are probably unobtainable due to ancillary age-related diseases, but they provide an upper bound on what might be possible.

The proposed molecular pathway from mitochondrial ROS production to senescence leads to several simple predictions. ROS inhibitors may be able to delay or prevent age-related diseases. Telomeric interventions may be able to prevent or cure age-related diseases. Senescence interventions, including senolytics and senomorphics, may be able to prevent or treat age-related diseases. However, by the duality hypothesis, all such interventions must be careful not to interfere with any vital life-enhancing role. Interfering with genes that effect energy and nutrient uptake is somewhat promising because the life enhancing role of increased energy uptake in the evolutionary environment no longer exists. Similarly, interfering with pro-aging genes that also influence development is promising as such genes may no longer play a role in the developed organism.

Other approaches to aging that may initially appear unrelated to the mechanisms proposed here are worth considering. If the molecular pathway proposed here is correct it should largely be possible to align the other approaches with this pathway. This is done to good effect in Appendix B.

6 Discussion

The evidence for and against hypotheses on the extended phenotype of the mitochondrion (HEPM) is presented in one place in Appendix C.

Overall, as seen throughout this paper, the available evidence appears to strongly support HEPM. In reaching this conclusion it is important to look not just at the number of pieces of evidence, but to weigh the strength of the evidence for and against. Some of the strongest evidence for HEPM relates to ROS and senescence. Mitochondria have been suspected to drive the process that leads to cellular senescence[13], but the reason why has been elusive. Another important area is in understanding the seeming intentionality of many age-related diseases, and being able to explain why humans live so much longer than mice. Here selection of the nuclear genes has prevented jumping to the conclusion that it is intentional, but if the process is being driven by kin selection of mitochondrial genomes, intentionality suddenly becomes plausible.

HEPM offers new biological and biomedical insight by providing answers to the important questions of how did sex evolve, and how is sex evolutionarily stable, as well as why eukaryotes are mortal, and explaining the root cause of many age-related diseases. These have been thorny questions that for a long time have gone unanswered.

The mitochondrial genome is engaged in the ultimate crossbreeding experiment for its own benefit. This involves the crossbreeding of nuclear genomes to produce a more beneficial environment within which for it to reside. The mitochondrial genome achieves this crossbreeding through the production of ROS, which renders the organism mortal, and leaves sex as the only long term option for the nuclear genome to pass on, at least some of, its genes. The results of this crossbreeding

What explains facultative sexual reproduction such as occurs in some plants and fungi?
How does HEPM apply to plants with their energy producing plastid genome?
Might mitochondrial-derived peptides play the role of rejuvenating the cell during meiosis?
What is happening with the mitochondrial ribosome of bdelloid rotifers? Section 2.3.1.
Does HEPM imply changes to models of species and speciation? Sections 2.4 and 3.2.
Does phosphorylated ATM level correlate with senescence or not? Section 4.5.3.

Table 2: Some questions raised by hypotheses on the extended phenotype of the mitochondrion.

experiment are all the varied eukaryotic forms that now exist, and within which trillions of trillions of mitochondrial genomes now reside.

Treating the mitochondrial genome as a selfish replicator offers a different way of looking at the eukaryotic cell, and with it new understanding. Sex might have evolved as a means for the proto-mitochondria to propagate itself into a more and more competent host. Thus creating the defining advantage for eukaryotes relative to prokaryotes: meiosis. Aging and mortality exist at least in part as a means of ensuring meiosis occurs at a near to optimal frequency for the species. And the whole eukaryotic cell can be viewed as the extended phenotype of the mitochondrial genome. This isn't the only way the eukaryotic cell should be viewed, but it adds an interesting new perspective.

Arguments against HEPM appear muted. The strongest argument against HEPM appears to be that mild ROS exposure, or an increase in ROS production, can sometimes extend lifespan. This though is by no means universal. Furthermore, ROS also play a role as signaling molecules[78], and it might be hypothesized that the increased ROS activates the cell's antioxidant system, leading to reduced cytosolic ROS over the long term. A pathway like this is known to occur for caloric restriction in the yeast *Saccharomyces cerevisiae* and germline signaling in *Caenorhabditis elegans*. A second argument against HEPM is the resetting of replicative age in daughter cells, again in the yeast *S. cerevisiae*. But so far, this is known to occur in just one species. And here the failure to mate in the presence of mating pheromone leads to apoptosis. This creates a limit on lifespan that applies even to daughter cells.

If correct, HEPM raises a number of new questions and enhances the importance of some existing questions as listed in Table 2.

If it was possible to increase human lifespan to, say, 150 or 200 years, this would, assuming no change in female reproductive lifespan, result in a doubling or tripling of the planet's population. This would have many serious social and environmental implications. Despite this it appears desirable. Otherwise why else would we today be investing heavily in finding cures to many age-related diseases through one-disease-at-a-time approaches. It is just that the one-disease-at-a-time approaches are only likely to be weakly effective, while targeting the core mechanism of aging has the potential to make major gains in healthspan and lifespan.

HEPM makes an important clinical prediction: ROS inhibitors, telomeric interventions, and senescence interventions including senolytics and senomorphics, may be capable of preventing, treating, or curing many age-related diseases. The heavy burden of age-related diseases strongly argues for a Manhattan or Apollo project-like effort to better understand the fundamental biology of aging and to invest in the development and clinical trial of senolytics, senomorphics, telomeric interventions, and ROS inhibitors so as to delay, prevent, treat, and cure these age-related diseases.

7 Materials and methods

For the bioinformatic analysis of rRNA large subunit sizes in section 2.3.1, annotated mitochondrial genomes from the NCBI Reference Sequence Database (RefSeq) release 214 were used. See Supplement 1 for a copy of this data[193]. Of these genomes, 13,645 were identified as being annotated with a name indicating the ribosomal large subunit. Reported sizes and maximum possible sizes were both computed from the annotations.

For the bioinformatic analysis of mitochondrial gene frequencies in Appendix A, the same 13,959 quality filtered genomes were used. After filtering for data quality the 14,062 genomes were reduced to 13,959 genomes. Different names used in the annotations for the same orthologous genes were mapped to the most common name. This process may have missed some annotated names that only occurred once, and orthologs that have split into multiple separate genes. The frequency of different genes was then computed.

See Supplement 2 for the software used for both analyses[194].

Appendices

A Mitochondria

The mitochondrion is an organelle present in the vast majority of eukaryotic cells. The mitochondrion contains a double membrane. Each membrane is a phospholipid bilayer with embedded proteins. The space between the inner and outer membranes is termed the intermembrane space. The space within the inner membrane is termed the matrix.

Somewhere in the range of 100 to 500 mitochondria are found in a typical cell[195]. The mitochondria in the cell are capable of undergoing processes of fission (splitting) and fusion (joining). Mitochondria may engage in these dynamics as part of a quality control mechanism that also involves autophagy[196].

The structure of a typical mitochondrion is illustrated in Figure 3.

Mitochondria nearly always have their own circular double-stranded DNA genome, commonly referred to as mtDNA. This genome is much smaller than the nuclear genome both in terms of the number of genes encoded, and in terms of the number of base pairs.

mtDNA differs far more between species than within species. Indeed, it is hypothesized that all the mitochondrial genomes found in humans today descended from a single mitochondrial "Eve" that existed perhaps 200,000 years ago[197].

Multiple copies of the same mtDNA genome exist within a single eukaryotic cell. A typical cell might have around 5,000 mtDNA copies contained within its mitochondria[198].

The mtDNA genome almost invariably includes genes for the large and small subunits of the mitochondrial ribosome and usually all of the corresponding mitochondrial tRNA genes. The

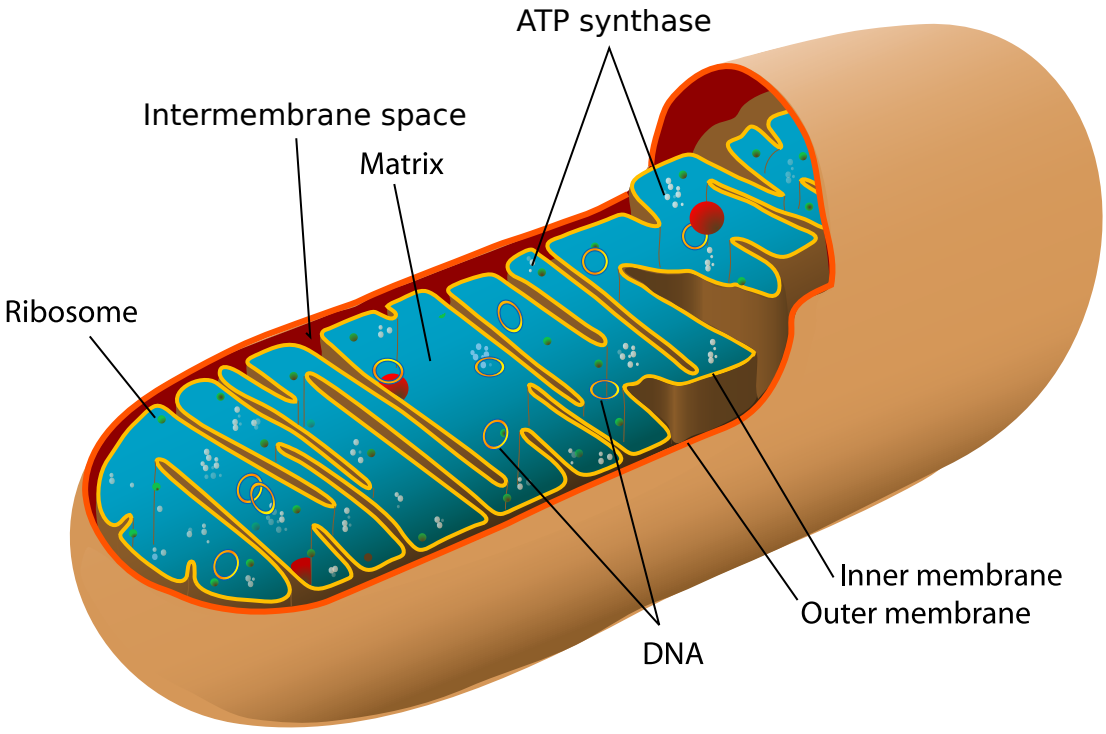

Figure 3: Structure of a typical mitochondrion.

CYTB	99.6%
COX1	99.5%
COX3	99.4%
ND5	99.0%
ND4	99.0%
ND2	99.0%
COX2	99.0%
ND6	98.9%
ATP6	98.9%
ND3	98.9%
ND1	98.9%
ND4L	98.7%
ATP8	95.1%
ATP9	9.7%
RPS3	7.5%
RPS12	5.4%
RPL16	4.9%
ND9	4.6%
ND7	4.5%
RPS4	4.5%

Table 3: Estimated frequency of the top 20 mtDNA protein coding genes from an analysis of 13,959 RefSeq mitochondrial genomes. Due to vagaries in the names used for orthologous genes, frequencies are likely to be slight under-estimates. For further details see section 7.

remaining mtDNA genes vary to some extent from species to species. These remaining genes nearly always include genes for components of the electron transport chain (COX, ND, and CYTB genes) and ATP synthase (ATP genes). Occasionally genes for mitochondrial ribosomal proteins (RPL and RPS genes) are also present. This is shown in Table 3. Most species' mitochondria comprise the same 13 protein coding genes, but the sequence making up each gene will vary between species.

The vast majority of mitochondrial proteins are not encoded by the mtDNA, but by the nuclear genome[110], and are directed to the mitochondria by the presence of a mitochondrial targeting presequence that is cleaved off.

The mitochondria are the locations of the energy producing reactions of the cell. The citric acid cycle turns pyruvate and water into CO_2 and in so doing produces the cofactors NADH, $FADH_2$, and GTP. The electron transport chain oxidizes NADH and $FADH_2$ releasing energy which is used to pump H^+ from the matrix to the intermembrane space. ATP synthase then uses the resulting H^+ electrochemical gradient to produce ATP from ADP.

mtDNA is normally maternally inherited. Various mechanisms exist to prevent the paternal inheritance of mtDNA in most species[199].

Base pairs in mitochondrial genes evolve 10 times more rapidly than base pairs in nuclear genes, but because the mtDNA coding regions are roughly $\frac{1}{2{,}000}$th the length of nuclear DNA coding regions, the mitochondrial genome effectively evolves 200 times more slowly than the nuclear genome. To

be precise, humans and chimpanzees are estimated to have diverged $T = 6.7\times10^6$ years ago[59]. Comparing human and chimpanzee mtDNA, the non-synonymous substitution rate of protein coding genes is 2×10^{-9} substitutions per site per year[200]. The rate of substitution for the mtDNA rRNA genes is somewhat higher. For synonymous sites the substitution rate is 3×10^{-8}[200]. These substitution rates should be compared to the nuclear DNA non-synonymous and synonymous substitution rates of protein coding genes of around 2×10^{-10} and 9×10^{-10} substitutions per site per year respectively[201][Supplement S23, site weighted K_a and K_s values divided by $2T$].

The somatic mutation rate of the mitochondrial genome is around 2×10^{-7} mutations per base pair per year based on mutational accumulation in aged humans[202][1.9×10^{-5} mutations divided by a mean age of 83 years]. It is hypothesized that the female germ line contains quiescent template mitochondria that are protected from this high rate of mutation[203].

The human mitochondrial genome contains 16,568 base pairs. In addition to genes for the large and small subunits of the mitochondrial ribosome, it contains the standard 22 tRNA genes, 11 electron transport chain genes, and 2 ATP synthase genes. The gene content of the human mitochondrial genome is identical to that of most other organisms, although in terms of base pairs fungal and plant mitochondrial genomes are substantially larger.

B Other approaches to aging

This appendix reviews other approaches to aging, and shows that they can largely be aligned with the pathway proposed in section 4.5. Multiple mechanisms for some of these other approaches have been suggested. In reviewing these other approaches proposed mechanisms that align with the pathway proposed in section 4.5 are considered.

B.1 Caloric restriction

The lifespan extending effects of caloric restriction have been discussed in section 4.2. There it is hypothesized that caloric restriction results in a lower rate of ATP and thus ROS production, extending lifespan.

B.2 Down-regulation of the insulin/IGF-1 signaling pathway

Insulin signals to the organism the availability of glucose energy that should be taken up by cells. Insulin-like growth factor 1 (IGF-1) stimulates cell growth, proliferation, and survival[204]. The down-regulation of the insulin/IGF-1 signaling pathway has been proposed as an anti-aging intervention[205].

The mitochondrial genome seeks to optimize the fitness of the species. It does this by enforcing mortality on the organism. Fitness will be maximized if organism lifespans are kept short. If the lifespan is too short however there will be insufficient time for reproduction to occur. If the organismal environment has little energy, it will take longer for the organism to grow and reproduce, and it might be expected that there would be a more permissive mitochondrial mandate regarding

experiment type	experiment id.	ctl-1	ctl-3
microchip	NCBI GEO DataSets GSE106672[210]	2.4	2.5
RNA-Seq	NCBI GEO DataSets GSE111338	1.5*	2.8
NRA-Seq	NCBI GEO DataSets GSE70117[211] at 15°C	1.5	1.8
NRA-Seq	NCBI GEO DataSets GSE70117[211] at 25°C	2.9	2.6
RNA-Seq	NCBI GEO DataSets GSE67975[212]	1.6	4.2

Table 4: N-fold change in catalase mRNA of *C. elegans* daf-2 mutants versus control. * - mapping locus includes both ctl-1 and ctl-2.

the lifespan of the organism. This may be why caloric restriction works. Similarly, if the organism is tricked into believing it is in a low energy environment, it might be expected to exhibit an increased lifespan. As discussed below, this appears to be the case: down-regulation of insulin/IGF-1 signaling increases lifespan.

Caenorhabditis elegans has a single insulin/IGF-1 receptor gene, daf-2. daf-2 mutants show increased lifespan[206]. daf-2 mutants exhibit a change in gene expression compared to the wild-type that is mediated by several transcription factors. This includes daf-16 up-regulation, a forkhead subclass O (FOXO) transcription factor[205]. In *Drosophila melanogaster* inhibition of insulin/IGF-1 signaling or increasing FOXO increases lifespan[205]. In mice there is a negative correlation between IGF-1 levels and lifespan[205]. Finally, small dogs have a mutation that decreases IGF-1 levels and live longer[205].

One possible mechanism through which daf-2 mutants might extend lifespan is by causing the cell to adapt to the low energy environment by reducing the cellular demand for ATP which would then reduce ROS production. The reduction in ROS would then reduce the rate of telomeric DNA damage. In this regard, daf-2 mutants are known to exhibit differentially expressed mRNA lipid, protein, and energy metabolism gene families[207].

A second possible mechanism by which daf-2 mutants extend lifespan might be through a reduction in the level of H_2O_2. This reduction might occur through the up-regulation of H_2O_2 reducing genes. Unlike humans, which possess a single catalase that is located in the peroxisome, *C. elegans* contains 3 catalase genes. ctl-1 is widely considered to be cytosolic[208], although WormBase WS286 lists its putative location as peroxisomal and mitochondrial[209]. ctl-2 is peroxisomal[208]. ctl-3's location is uncharacterized[208], but predicted to be peroxisomal and mitochondrial in WormBase WS286. Mitochondrial and cytosolic catalases in particular can be expected to reduce cytosolic ROS levels and reduce telomeric damage. Up-regulation of these catalases in daf-2 mutants has been confirmed by examining the results from a few gene expression experiments as shown in Table 4.

B.3 Down-regulation of mTOR

The mammalian target of rapamycin (mTOR) kinase is an energy and nutrient sensor that stimulates growth and blocks autophagy when nutrients are plentiful[205].

The mTOR pathway has invoked considerable interest as a possible aging mechanism[213]. Inhibition of mTOR has been shown to significantly extend lifespan in a number of species[214].

Inhibition of mTOR down-regulates the production of multiple protein synthesis components, including ribosomes, initiation factors, and elongation factors[215]. Thus inhibition of mTOR will reduce the energy needs of the cell. Reducing the energy needs of the cell should reduce the amount of oxidative phosphorylation performed by the mitochondria, and hence reduce the production of ROS. In addition it has been shown that the inhibition of mTOR increases the translation of mitochondrial encoded oxidative phosphorylation subunits, which likely leads to few electrons transiting a given electron transport chain, an oxidized chain, reduced ROS production, and less ROS-mediated cellular damage[214]. Therefore lifespan extension by mTOR inhibition might be mechanistically linked to mTOR's role as a mitochondrial ROS inhibitor.

Down-regulation of mTOR has been associated with muscular atrophy and frailty[216]. This negative effect is consistent with the corollary to the duality hypothesis.

B.4 Up-regulation of AMPK

The AMP-activated protein kinase (AMPK) is activated when the AMP to ATP ratio rises[217]. Amongst other things activated AMPK inhibits mTOR and promotes mitochondrial biogenesis[218, 219]. This mitochondrial biogenesis includes production of mitochondrially encoded proteins[220].

Over-expression of the AMPK activator aak-2 in *C. elegans* has been shown to extend lifespan[221].

Both the inhibition of mTOR and increased mitochondrial biogenesis without a concomitant increase in the energy demands of the cell, might be expected to reduce ROS, and with it extend lifespan.

B.5 Up-regulation of sirtuins

Sirtuins are a family of NAD+ dependent deacetylases and ADP-ribosyltransferases[222]. Over-expression of the sirtuins SIRT1 and SIRT6 has been demonstrated to extend lifespan in various species[222].

Mice, unlike humans, express telomerase in somatic cells[223]. In mice SIRT1 expression correlates with telomere length and reduces age related telomere shortening[224]. In humans a single nucleotide polymorphism in SIRT1 correlates with telomere length and longevity[225].

SIRT6 deacetylates histone H3K9 promoting telomere stability by enabling telomere association with Werner syndrome ATP-dependent helicase (WRN)[226]. Mutations in WRN result in Werner syndrome, a disease exhibiting premature aging[227]. SIRT6 knockout mice exhibit hypersensitivity to H_2O_2[228]. SIRT6 is also believed to play a role in stimulating DSB repair, with more effective SIRT6 activity correlating with longer lifespan[229]. Finally, SIRT6 deficiency is associated with increased NF-κB signaling[230].

In summary, SIRT1 and SIRT6 may extend lifespan by affecting telomere length, and assisting in

telomere damage repair processes.

B.6 Manipulation of redox pathways

Mitochondrial thioredoxin reductase (TrxR) levels are elevated in long lived species of primates, rodents, and birds[231]. Disruption of Trx or TrxR shortens lifespan, increased Trx or TrxR expression can extend it, and allelic variation in cytosolic TrxR has been associated with longevity in humans[232].

NADPH reduces TrxR, which then reduces Trx. The existence of reduced Trx is key to the reduction of peroxiredoxin (Prx), which enables Prx to reduce H_2O_2 to water, and more importantly reduced Trx inactivates the ASK1-JNK pathway that leads to the SASP as shown previously in Figure 2.

Trx can also be reduced by glutaredoxins, which are reduced by the oxidation of reduced glutathione (GSH)[232]. GSH is generated by glutathione reductase (GR), which is reduced by NADPH. Accordingly, acceleratingly aged mice and naturally aged mice and humans show decreasing levels of the antioxidants GSH and GR with age[233].

Thus, both by reducing H_2O_2 and through the production of reduced Trx which interferes with ASK1, increases in redox reduction pathways may extend lifespan.

B.7 Modulation of germline signaling

The removal of the germ cells in *C. elegans* significantly increases lifespan[205]. Countervailing this, the removal of the ovaries is correlated with increased all cause mortality in women[234].

In the case of *C. elegans*, germline loss appears to result in a bust of ROS in somatic tissues in early adulthood[235]. In response to this burst in ROS mitochondrial biogenesis is increased[235]. This increase in mitochondrial content could be hypothesized to lead to reduced ROS production over the long term, and increased lifespan.

Women undergo a gradual loss of germ cells as they age. The depletion of germ cells typically occurs earlier than death, and might represent a mechanism to ensure that resources are directed to viable offspring. For women, the presence of germ cells might thus cause the nuclear genes of the organism to seek to resist the aging process.

B.8 Enhanced autophagy

Elevated levels of autophagy occur in common with multiple lifespan extending interventions: reduced insulin/IGF-1 signaling, reduced mTOR signaling, germline removal, caloric restriction, and reduced mitochondrial respiration[236]. As such, autophagy is hypothesized as a common mechanism of aging, and interventions to enhance autophagy are hypothesized to extend lifespan. Mitochondrial mechanisms have been proposed here whereby each of these interventions may extend lifespan without having to invoke autophagy as an explanation. These proposed mechanisms might suggest that the link between autophagy and lifespan may be more correlative than causative.

Autophagy related 5 (ATG5) is a key gene of autophagy. The over-expression of ATG5 in mice enhances autophagy and extends lifespan[237]. ATG5 transgenic mice had the same food intake per body weight, but weighed slightly less, and so had less food intake overall[237].

The effect of autophagy could thus be correlative, or it could be to reduce the energy needs of the cell, thereby reducing mitochondrial respiration, and in this way extending lifespan.

B.9 Parabiosis

Continuous blood exchange between an older and a younger animal, heterochronic parabiosis, improves cellular proliferation in the older animal, and reduces the lifespan of the younger animal[238, 239]. mRNA levels of the senescence markers p16 and p21 and SASP genes are reduced in the older animal as a result of heterochronic parabiosis[240].

A possible mechanism for heterochronic parabiosis is through the modulation of one or more endocrine factors making up the SASP. Both the SASP factors IL-6 and TNF appear capable of exerting endocrine effects[241, 242].

B.10 Metformin

Metformin is the first line drug for the treatment of type 2 diabetes[243]. Metformin is also associated with a 30-50% reduction in the risk of cancer among type 2 diabetes patients[244]. Metformin extends lifespan in *Caenorhabditis elegans* and some strains of *Mus musculus*, but not in *Drosophila melanogaster*[245]. Metformin is proposed to be tested as a drug to increase healthy human lifespan in the TAME trial[246].

The precise mechanism by which metformin exerts its multiple effects has not been fully elucidated. One of several possibilities is that it reduces cytosolic ROS concentrations. Metformin has been shown to inhibit complex I of the electron transport chain[247, 248]. A reduction in complex I activity should result in a reduction in the activity of subsequent electron transport chain units, and a reduction in cytosolic ROS. A related mechanism of action for metformin is through the activation of AMPK which is also hypothesized here to reduce ROS[249].

Metformin potentially illustrates the role of mitochondrial ROS production in mortality, and the potentially beneficial effects of ROS inhibition.

B.11 Epigenetic reprogramming

The loss of epigenetic information such as DNA and histone methylation and histone acetylation patterns has been proposed to occur as a part of the aging process[250]. As such, epigenetic reprogramming may be able to treat certain age-related diseases.

It is possible to construct a pathway from mitochondrial ROS production to the loss of epigenetic information via the displacement of SIRT1, which plays a role in the histone deacetylation that maintains epigenetic silencing, and is also involved in DSB repair[251]. ROS are assumed to create

DSBs and the recruitment of SIRT1 to this damage prevents it from playing its role in epigenetic silencing. However these arguments are currently only speculative. If proven correct, the loss of epigenetic information might turn out to be a fundamental mechanism of aging of mitochondrial origin according to the classification scheme proposed here. Otherwise the loss of epigenetic information might be viewed as having occurred infrequently in the evolutionary environment and thus being classified as an ancillary mechanism of aging.

C Review of evidence

This appendix collects in one place both the evidence for and against hypotheses on the extended phenotype of the mitochondrion (HEPM) in Tables 5 and 6 and in Table 7 respectively. Evidence for the hypotheses that exists, but is not discussed in this paper has been omitted. On the other hand, all known evidence against the hypotheses has been discussed and presented. Despite this, the evidence for the hypotheses appears substantially greater than the evidence against.

Not every one of the answers to the questions in Tables 5 and 6 will likely be correct. And some of these questions already have one or more existing plausible answers. However most of these are single point theories that address one question. By the principle of parsimony there is considerable advantage to replacing them by a single unifying theory.

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Conflict of interest disclosure

The author declares they have no financial conflicts of interest in relation to the content of this manuscript.

Supplements

Supplement 1 - Mitochondrial genome sequence data.
`https://doi.org/10.5281/zenodo.7901579`

Supplement 2 - Mitochondrial genome analysis software and results.
`https://doi.org/10.5281/zenodo.7901623`

H1	How can active germ line replicators combine? Section 1.4
H1	How did sex evolve? Section 2.1.
H1	Why is evidence for eukaryotes prior to mitochondrial symbiosis lacking? Section 2.1.
H1	Why do eukaryotes have multiple nuclear chromosomes? Section 2.1.
H1	Who benefits from sex? Section 2.2.
H1	What did the mitochondrion get in return for its symbiotic bargaining power? Section 6.
H2	Why is the mitochondrial genome of the bdelloid rotifers so anomalous? Section 2.3.1.
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H2	Why are there so few ancient asexual eukaryotes? Sections 2.3.1 and 2.4.
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H3	Why are eukaryotes mortal? Section 3.
H3	Why do unicellular eukaryotes appear to engage in apoptosis? Section 3.
H3	Why is lifetime energy consumption per unit mass constant across species? Section 3.1.
H3	Why is obesity correlated with shortened lifespan? Section 3.1.
H3	Why does the Galápagos tortoise live so much longer than insects? Section 3.2.
H3	Can selection operate at the level of the species? Section 3.2.
H3	Why does failing to mate in the presence of pheromones induce apoptosis? Section 4.3.
H3	Why does recombination reset replicative age in yeast? Section 4.3.
H3	Why do humans live so much longer than mice? Section 4.4.4.
H3	Why is the mortality rate so low throughout the reproductive lifespan? 4.4.4.
H3	Why does down-regulation of insulin/IGF-1 signaling extend lifespan? B.2.
H4	Why is age reset by sex? Section 4.
H4	Why do most animal species exhibit organismal senescence? Section 4.4.
H4	Why does the thymus involute? Section 4.4.2.
H4	Why do so many human diseases exhibit the hallmarks of intentionality? Section 4.4.3.
H4	Why do humans and mice experience many of the same causes of death? Section 4.4.4.
H5	Why do apoptotic death effectors also perform some vital function? Section 4.1.
H5	Why is senescence beneficial for development and wound healing? Section 4.4.1.
H5	Why is the ATM mediated DDR both a pro-aging and life-giving signal? Section 4.5.2.
H5	Why does down-regulation of mTOR extend lifespan and muscular atrophy? Section B.3.
H6	Why do mitochondria appear to play a key role in apoptosis? Section 3.
H6	Why is catalase usually only found in the peroxisome? Section 4.2.
H6	Why do mutator mice show increased ROS as they age? Section 4.2.
H6	Why does a mitochondrially targeted catalase reduce cardiomyopathy? Section 4.2.
H6	Why does ROS inversely correlate with lifespan across species? Section 4.2.
H6	Why does overexpression of antioxidant genes increase lifespan? Section 4.2.
H6	Why does deletion of antioxidant genes decrease lifespan? Section 4.2.
H6	Why does exposure to antioxidants increase lifespan? Section 4.2.

Table 5: Primary hypotheses and questions answered or informed by hypotheses on the extended phenotype of the mitochondrion.

H6	Why does mitochondrial haplogroup D4a correlate with extreme lifespan? Section 4.2.
H6	Why does caloric restriction extend lifespan? Section 4.2.
H6	Why does deletion of SOD1 or SOD2 reduce RLS in yeast? Section 4.3.
H6	Why does deletion of SOD1 or SOD2 reduce CLS in yeast? Section 4.3.
H6	Why does overexpression of SOD1 or SOD2 increase CLS in yeast? Section 4.3.
H6	Why is ROS implicated in thymic involution? Section 4.4.2.
H6	Why does iron negatively influence lifespan? Section 4.5.1.
H6	Why do telomeres contain the sequence AGGG? Section 4.5.1.
H6	Why are telomere DSBs irreparable? Section 4.5.2.
H6	Why does telomere damage increase with age? Section 4.5.2.
H6	Why do shortened telomeres correlate with age? Section 4.5.2.
H6	Why do shortened telomeres correlate with mortality risk? Section 4.5.2.
H6	Why does ROS accelerate telomere shortening? Section 4.5.2.
H6	Why does SOD3 reduce the rate of telomere shortening? Section 4.5.2.
H6	Why don't eukaryotes possess circular chromosomes? Section 4.5.2.
H6	Why do daf-2 mutants have increased catalase expression? Section B.2.
H6	Why does the inhibition of mTOR extend lifespan? Section B.3.
H6	Why does the activation of AMPK extend lifespan? Section B.4.
H6	Why does enhancement of autophagy extend lifespan? Section B.8.
H6	Why does metformin reduce the risk of cancer? Section B.10.
H6	Why does metformin extend lifespan in *C. elegans*? Section B.10.
H7	Why do mitochondria appear to play a key role in senescence? Section 4.4.
H7	Why do senescent cells exhibit increased mitochondrial ROS? Section 4.4.1.
H7	Why does the SASP exist? Section 4.4.1.
H7	Why does a reduction in the number of mitochondria attenuate the SASP? Section 4.4.1.
H7	Why does senescence appear to spread once a threshold level is reached? Section 4.4.1.
H7	Why do antioxidants prevent senescent cell cycle arrest? Section 4.4.1.
H7	Why does electron chain disruption contribute to senescence? Section 4.4.1.
H7	Why is the SASP implicated in thymic involution? Section 4.4.2.
H7	Why do so many age-related diseases involve senescence and the SASP? Section 4.4.3.
H7	Why does phosphorylated ATM level correlate with age? Section 4.5.3.
H7	Why is the p16 level considered a biomarker of age? Section 4.5.4.
H7	Why is p16 elevated in senescent cells? Section 4.5.4.
H7	Why does phosphorylated ATM arrest the cell cycle? Section 4.5.4.
H7	Why does phosphorylated ATM induce senescence associate genes? Section 4.5.5.
H7	Why does phosphorylated ATM induce the SASP? Section 4.5.6.
H7	Why does SIRT1 or SIRT6 over-expression extend lifespan? Section B.5.
H7	Why does Werner syndrome result in accelerated aging? Section B.5.
H7	Why does TRx and TrxR correlate with lifespan? Section B.6.
H7	Why do GSH and GR levels decline with age? Section B.6.
H7	Why does parabiosis modulate aging? Section B.9.

Table 6: Primary hypotheses and questions answered or informed by hypotheses on the extended phenotype of the mitochondrion (continued).

H3	Hydra are able to regenerate without sex?
H3	The replicative lifespan of yeast daughter cells is reset by mitosis. Section 4.3?
H6	Peroxisomal H_2O_2 should interfere with mitochondrial ROS signaling? Section 4.5.1.
H6	Mild ROS exposure increase lifespan? Section 4.2.
H6	Mutations that increase ROS sometimes increase lifespan? Section 4.2.
H6	Overexpression of SOD1 fails to increase RLS in yeast? Section 4.3.
H6	Iron supplementation extend CLS in yeast? Section 4.5.1.

Table 7: Primary hypotheses and evidence against hypotheses on the extended phenotype of the mitochondrion.

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