HHS Public Access

Author manuscript

Hum Genet. Author manuscript; available in PMC 2021 March 01.

Published in final edited form as:

Hum Genet. 2020 March ; 139(3): 381–399. doi:10.1007/s00439-020-02119-5.

Mitonuclear genomics and aging

Joseph C. Reynolds[1], **Conscience P. Bwiza**[1], **Changhan Lee**[1,2,3]

[1]Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA 90089, USA

[2]USC Norris Comprehensive Cancer Center, Los Angeles, CA 90089, USA

[3]Biomedical Sciences, Graduate School, Ajou University, Suwon 16499, South Korea

Abstract

Our cells operate based on two distinct genomes that are enclosed in the nucleus and mitochondria. The mitochondrial genome presumably originates from endosymbiotic bacteria. With time, a large portion of the original genes in the bacterial genome is considered to have been lost or transferred to the nuclear genome, leaving a reduced 16.5 Kb circular mitochondrial DNA (mtDNA). Traditionally only 37 genes, including 13 proteins, were thought to be encoded within mtDNA, its genetic repertoire is expanding with the identification of mitochondrial-derived peptides (MDPs). The biology of aging has been largely unveiled to be regulated by genes that are encoded in the nuclear genome, whereas the mitochondrial genome remained more cryptic. However, recent studies position mitochondria and mtDNA as an important counterpart to the nuclear genome, whereby the two organelles constantly regulate each other. Thus, the genomic network that regulates lifespan and/ or healthspan is likely constituted by two unique, yet co-evolved, genomes. Here, we will discuss aspects of mitochondrial biology, especially mitochondrial communication that may add substantial momentum to aging research by accounting for both mitonuclear genomes to more comprehensively and inclusively map the genetic and molecular networks that govern aging and age-related diseases.

Introduction

Mitochondria are frequently labeled “the powerhouse” of the cell, reflecting their role as the primary bioenergetic source, yet their biological functions are remarkably extensive. They are increasingly being appreciated for their role in sensing environmental cues and coordinating/communicating adaptive responses to other cellular compartments, including the nucleus. A wide range of cellular functions are now known to be regulated by mitochondria, including multiple age-related processes, such as metabolism, unfolded protein response, autophagy, and inflammation (Chandel 2015; Hill et al. 2018; Melber and Haynes 2018; Quirós et al. 2016; Sun et al. 2016).

Changhan Lee, changhan.lee@usc.edu.

Conflict of interest C.L. is a consultant and shareholder of CohBar, Inc. All other authors declare no competing interests.

Mitochondria are cogently thought to originate from endosymbiotic bacteria that emerged as early as 1.5 billion years ago (Martijn et al. 2018; Quirós et al. 2016; Spang et al. 2015; Sunnucks et al. 2017). Mitochondria have retained many of their inherited prokaryotic properties, including a circular genome (mitochondrial DNA; mtDNA) with a unique genetic code, formylation of mitochondrial proteins, and binary fission and fusion. The modern mtDNA is estimated to be considerably reduced from the primeval bacterial genome, resulting from loss and lateral transfer to the nuclear genome. The survival advantage of maintaining two genomes is unclear, but the co-evolution of mitochondrial and nuclear (mitonuclear) genomes likely required, and likely still requires, continuous adaptation to each other to establish a unified singular genetic system.

During the past century, remarkable progress has been made in unveiling the mechanisms of aging. Genetic and molecular pathways that regulate healthspan and lifespan have been identified in various model organisms, providing a rich knowledge base (Longo et al. 2015; Lopez-Otin et al. 2013, 2016; Singh et al. 2019). However, the focus on longevity pathways has been nuclear-centric and all known longevity genes are nuclear-encoded. In this review, we will discuss key aspects of the mitochondrial genome and mitonuclear communication, which may add additional momentum to aging research by accounting for both genomes to more comprehensively and inclusively map the genetic and molecular networks that govern lifespan and/or healthspan.

Mitochondria: origin and genome

Mitochondria presumably originate from endosymbiotic alpha-proteobacteria (Sagan 1967) and continue to possess multiple prokaryotic remnants including their own unique circular genome and genetic code. While the specific evolutionary origin of mitochondria remains debatable, the integration of two free-living organisms likely required dynamic communication and coordination (Lane 2017; Youle 2019). The mitochondrial DNA (mtDNA) and the nuclear DNA conceivably have been evolving for over 1.5 billion years. It is estimated that a considerable amount of the original bacterial genome has been lost or transferred to the nuclear genome (Bock 2017). Notably, mitochondria-to-nucleus gene transfer still occurs in modern eukaryotic cells (Ju et al. 2015).

The mitochondrial genome has been traditionally described to encode for 37 genes; 13 proteins (mRNAs), 2 ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs). All 13 proteins are components of the mitochondrial respiratory chain (MRC). For example, of the five complexes involved in oxidative phosphorylation (OXPHOS), complex I is composed of 45 polypeptides, of which seven are encoded in the mtDNA (Wallace 2010). Only complex II is entirely assembled from nuclear-encoded subunits. The stoichiometry of the MRC subunits is critical for OXPHOS (Milenkovic et al. 2017) and the mitochondrial and cytosolic translation of the MRC components are tightly coordinated (Couvillion et al. 2016). Mitochondrial-encoded rRNAs and tRNAs are exclusive to mitochondrial translation; nuclear-encoded tRNAs are imported into mitochondria in a species-specific manner (Rubio et al. 2008; Salinas-Giegé et al. 2015; Schneider 2011).

The regulation of the mitochondrial genome also reflects its prokaryotic ancestry. While nuclear DNA undergoes replication during cell division, mtDNA replication occurs independently of cell cycle. The majority of the components for mtDNA replication are imported nuclear-encoded proteins, including the catalytic subunit of mtDNA polymerase (POLGA), and the replicative mitochondrial helicase (TWINKLE) (Cermakian et al. 1996; Tyynismaa et al. 2004). Both the heavy and light strands of mtDNA contain genes, which are transcribed from three promoters; two are on the heavy chain (H1 and H2) and one is on the light chain (L) (Mercer et al. 2011; Shokolenko and Alexeyev 2017). A single subunit RNA polymerase transcribes mitochondrial genes, while translation requires mitochondrial-specific ribosomes using a distinct genetic code (Faye and Sor 1977; Kelly and Lehman 1986; Masters et al. 1987; Ringel et al. 2011). The H2 and L promoters transcribe almost the entire mitochondrial genome as a single polycistronic transcript. Genes in mtDNA lack introns and levels of unprocessed transcripts are low, indicating highly active co-transcriptional processing (Gustafsson et al. 2016). Notably, mitochondrial genes are typically flanked by tRNAs, which are then cut to produce individual transcripts (Anderson et al. 1981; Falkenberg et al. 2007; Mercer et al. 2011; Ojala et al. 1981). Interestingly, the mitochondrial transcription machinery is considered to have originated from the endosymbiotic alpha-proteobacteria that eventually became replaced with bacteriophage-derived factors (Gustafsson et al. 2016; Shutt and Gray 2006).

The mitochondrial genome is grouped and packaged in a nucleoid, which consists of DNA-binding proteins. Nucleoid architecture plays an important role in maintenance and transcription (Gilkerson et al. 2013; Kanki et al. 2004; Mercer et al. 2011), in which the mitochondrial transcription factor A (TFAM) is considered a key structural component (Kaufman et al. 2007). The overexpression and reduction of TFAM both affect mtDNA compaction level and interfere with mitochondrial function (Ekstrand et al. 2004). Additionally, TFAM deficiency has been shown to enhance nuclear DNA repair under chronic genotoxic stress by inducing a protective signaling response (Wu et al. 2019).

Mitochondrial-derived peptides

The human genome project annotated genes that encode for proteins generally > 100 amino acids (International Human Genome Sequencing 2004), leaving shorter peptides largely unknown. More recently, peptides that are encoded as short open reading frames [ORFs; a.k.a small ORFs (smORFs)] have been increasingly identified in the nuclear and mitochondrial genomes (Ingolia et al. 2014; Saghatelian and Couso 2015). Such polycistronic genes (i.e., genes-within-genes) have been traditionally thought to exist in prokaryotes to allow genomic compaction (Williams et al. 2005). Additionally, recent technological innovations in computational biology, sequencing, and proteomics have revealed a much larger portion of the genome that is transcribed and translated than was originally understood (Andrews and Rothnagel 2014; Rothnagel and Menschaert 2018; Ruiz-Orera and Albà 2019; Saghatelian and Couso 2015). In fact, accumulating evidence indicates that transcripts that harbor sORFs have been erroneously annotated as non-coding (Bazzini et al. 2014; Deng et al. 2018; Galindo et al. 2007; Ji et al. 2015; Kondo et al. 2007, 2010; Magny et al. 2013; Makarewich and Olson 2017; Nicholas et al. 2014; Yeasmin et al. 2018). It is estimated that thousands of nuclear-encoded sORFs that yield bioactive peptides

exist, enriching our proteome considerably (Raj et al. 2016). Several sORFs have been functionally described with diverse biological roles, including development (Chanut-Delalande et al. 2014; Kondo et al. 2010), DNA repair (Slavoff et al. 2014), muscle function (Bi et al. 2017), and immunity (Jackson et al. 2018).

Mitochondria have been known to code for 13 mRNAs, which are all components of the oxidative phosphorylation complexes, 22 tRNAs, and 2 rRNAs. However, recent studies have shown that mtDNA also encodes for previously unknown sORFs that yield biologically active peptides, collectively referred to as mitochondrial-derived peptides (MDPs) (Cobb et al. 2016; Hashimoto et al. 2001a, b; Lee et al. 2013, 2015). Currently, there are eight distinct MDPs that have been published (Hill et al. 2018): humanin (Guo et al. 2003; Hashimoto et al. 2001b; Ikonen et al. 2003), SHLP1-6 (Small Humanin-Like Peptide 1–6) (Cobb et al. 2016), and MOTS-c (Mitochondrial Open reading frame of the Twelve S rRNA type-c) (Lee et al. 2015).

The first MDP to have been detected at the protein level with functional description is humanin, which is a 24-amino acid peptide encoded within the 16S rDNA of mitochondria (Hashimoto et al. 2001a, b). Humanin has cytoprotective roles, including (1) enhancing resistance against Alzheimer's disease (AD)-related toxins (e.g., β-amyloid)(Hashimoto et al. 2001b), (2) anti-apoptotic effects by directly inhibiting BAX (Guo et al. 2003) and by downregulating p38 MAP kinase (Wang et al. 2005), and (3) by binding to insulin-like growth factor-binding protein-3 (IGFBP-3) and improving cell survival (Ikonen et al. 2003). Notably, humanin can be negatively regulated by IGF-1 and is positively correlated with longevity in mice and humans (Lee et al. 2014).

Humanin also plays a protective role in several pathological conditions. Humanin has been shown to be cardioprotective against myocardial ischemia–reperfusion (MI-R) injury by AMPK-endothelial nitric oxide synthase-mediated signaling and regulation of apoptotic factors (Muzumdar et al. 2010), reducing oxidative stress and promoting mitochondrial structural integrity (Klein et al. 2013), and reducing mitochondrial ROS levels and oxidative stress by targeting complex I (Thummasorn et al. 2016, 2018). Reduced age-related myocardial fibrosis was observed in 18-month old female mice that were treated with a humanin analog (HNG; 4 mg/kg, 2x/week, intraperitoneal injections) for 14 months (Qin et al. 2018b). Further, humanin protected human aortic endothelial cells against oxidized LDL-induced oxidative stress (Bachar et al. 2010), and preserved endothelial function in hypercholesterolemic ApoE-deficient mice (Oh et al. 2011). Notably, in humans, circulating humanin levels were negatively correlated with coronary endothelial function (Widmer et al. 2013), whereas higher levels of humanin were detected in unstable carotid plaques (Zacharias et al. 2012). Humanin and its derivative (S14G-humanin; HNG) (Hashimoto et al. 2001a, b) also showed beneficial effects in neurodegenerative disease models, such as protection against scopolamine-induced learning and memory impairment in mice (Mamiya and Ukai 2001), β-amyloid-induced hippocampal long-term potentiation in rats (Guo et al. 2010) and mice (Zhang et al. 2009), and β-amyloid-induced memory impairment in mice (Tajima et al. 2005).

Following the discovery of humanin, additional sORFs within the 16S rDNA have been identified and termed SHLPs (small humanin-like peptides). There are 6 SHLPs (SHLP1-6), which have unique and redundant biological effects, including cellular proliferation, apoptosis, and mitochondrial metabolism (Cobb et al. 2016). For example, SHLP2 and SHLP3 have antiapoptotic effects and promote cellular survival, while SHLP6 induces apoptosis in both murine beta-cells and prostate cancer cells (Cobb et al. 2016). Interestingly, humanin and SHLP2 have chaperone activities that can prevent the misfolding of islet amyloid polypeptide (IAPP), a pathogenic process in the development of type 2 diabetes mellitus (T2DM) (Okada et al. 2017). In addition, lower levels of circulating SHLP2 were associated with an increased risk for prostate cancer in white, but not in black, men (Xiao et al. 2017).

Mitochondria have 2 rRNA genes (12S and 16S rRNA). Whereas humanin and the 6 SHLPs are encoded within the 16S rRNA, another MDP was identified from the 12S rRNA. MOTS-c (Mitochondrial ORF within the Twelve S rRNA type-c) is a 16 amino acid peptide that is expressed in multiple tissues and in circulation, indicating dual roles as an intracellular and endocrine factor (Lee et al. 2015), a characteristic shared with humanin and SHLP1-6. Notably, MOTS-c expression is lost in cells with selective depletion of mtDNA (using low-dose chronic ethidium bromide; HeLa-ρ0) or mtRNA (using actinonin) without affecting the nuclear counterparts (Lee et al. 2015). In fact, the discovery of MOTS-c was inspired by the discovery that over 75% of mRNAs induced upon interferon activation in human myeloblasts mapped back to mitochondrial 12S and 16S rDNA loci (the paper didn't identify specific genes) (Tsuzuki et al. 1983). Notably, NUMTs that are identical to the mtDNA-encoded MOTS-c sequence are not found, although there are few similar sequences. This is consistent with the NCBI database, whereby only the mtDNA sequence for MOTS-c, and none of the NUMTs, have been recorded as mRNAs (Lee et al. 2015). Further, small mtRNAs (annotated as non-coding) exclusively map to mtDNA sequences, rather than NUMTs (Mercer et al. 2011; Pozzi and Dowling 2019). Whereas tissue-specific abundance of small mtRNA levels is strongly associated with mtDNA content, no association was observed with NUMT levels across six vertebrate species (Pozzi and Dowling 2019). MOTS-c expression requires cytosolic ribosomes because translation using the mitochondrial genetic code would lead to tandem start and stop codons (Lee et al. 2015). Although the specific mechanisms of mitochondrial nucleotide export are unknown, VDAC oligomers can form pores to secrete mtDNA fragments (Kim et al. 2019a), which have been increasingly appreciated as an adaptive mitochondrial stress response (Ingelsson et al. 2018; Trumpff et al. 2019; Yousefi et al. 2008). Upon leaving the mitochondria, it is plausible that the transcript may be translated using mitochondria-associated cytoplasmic ribosomes (Williams et al. 2014), thereby conferring mitochondrial specificity. Even then, it is possible that some NUMTs could encode for peptides, or express regulatory RNA, which would add another layer to the understanding of the evolution of our genomes as the origin of such sequences would still be mitochondrial.

Emerging studies continuously unveil the functions of MOTS-c in a wide range of pathophysiological processes as summarized in (Table 1). MOTS-c regulates cellular metabolic homeostasis by coordinating cellular glucose, fat, and protein metabolism. The key metabolic regulators AMPK and SIRT1 are required for several functions of MOTS-c,

including metabolism (Kim et al. 2018a; Lee et al. 2015; Lu et al. 2019b; Ming et al. 2016; Yan et al. 2019). In mice, MOTS-c has been shown to (1) enhance insulin sensitivity, largely by targeting skeletal muscle glucose metabolism (Lee et al. 2015), (2) promote white fat browning and brown fat activation in ovariectomized mice and mice exposed to cold (Lu et al. 2019a, b), (3) reduce fat mass, plasma lipid, and adipocyte size, while enhancing the lipid catabolism in ovariectomized mice (Lu et al. 2019b), in part, by increasing mitochondrial β-oxidation (Lee et al. 2015), (4) alleviate ovariectomy-induced bone loss by inhibiting RANKL-induced osteoclast formation (Ming et al. 2016) and osteoclastogenesis through osteocyte OPG/RANKL secretion (Yan et al. 2019).

In humans, MOTS-c has been implicated in different metabolic syndromes and diseases, including diabetes, cardiovascular diseases, and chronic kidney disease (CKD). Circulating MOTS-c levels were reported to be lower in obese male children and adolescents, especially in those who were insulin-resistant (Du et al. 2018). However, in adults, plasma MOTS-c levels were similar in both lean and obese subjects, but a positive correlation to insulin resistance was observed in lean subjects (Cataldo et al. 2018). These data suggest that MOTS-c levels change dynamically in a context-specific manner. Further, it is unclear if the levels of MOTS-c reflect a mechanistic contribution to the metabolic dysfunction, or a positive response to such metabolic perturbations. The role of MOTS-c in fat metabolism (Lee et al. 2015; Lu et al. 2019a, b) and its implication in obesity (Lee et al. 2015) are crucial since it is known that obesity is a risk factor for cardiovascular diseases (Eckel and Krauss 1998; Hubert et al. 1983). Moreover, adult patients with type 2 diabetes (Ramanjaneya et al. 2019a) showed reduced serum MOTS-c levels. Also, adult subjects with chronic kidney disease (CKD), in which diabetes and cardiovascular diseases are major risk factors, exhibited a decrease in MOTS-c levels in both serum and skeletal muscle (Liu et al. 2019).

Mitochondria dynamically communicate to other organelles, including the nucleus, to coordinate a myriad of vital cellular functions (Mottis et al. 2019; Quirós et al. 2016). Mitonuclear communication is especially interesting because it engages two organelles that hold independent genomes. However, traditionally, all known gene-encoded regulators of the mitonuclear genomes have been known to be nuclear-encoded. MOTS-c translocates to the nucleus in response to cellular stress in an AMPK-dependent manner to directly regulate adaptive nuclear gene expression by interacting with DNA and transcription factors (Kim et al. 2018a). MOTS-c provides evidence for cross-genomic regulation and extends the possibilities underlying the preservation of an independent mitochondrial genome.

Several of these mitochondrial-derived peptides may play a role in aging. SHLP2, humanin and MOTS-c all are positively correlated with longevity, and their levels decline with age in certain tissues (Cobb et al. 2016; Kim et al. 2017; Lee et al. 2015; Muzumdar et al. 2009). In both mice and humans, humanin is regulated through the GH/IGF-1 axis, which is a major conserved longevity pathway (Lee et al. 2014; Tatar et al. 2003). Humanin levels are lower in the short-lived GH-transgenic mice, yet higher in the long-lived GH-deficient mice (Lee et al. 2014). Interestingly, a MOTS-c polymorphism found in a Japanese population is related to exceptional longevity (Fuku et al. 2015; Zempo et al. 2016). At the functional level, MOTS-c can reverse age-dependent insulin resistance in mice (Lee et al. 2015). The

effect of MOTS-c on cellular metabolism is mediated, in part, by AMPK and SIRT1, which are key regulators of lifespan (Canto et al. 2009; Price et al. 2012).

mtDNA Diversity

Unlike the nuclear genome, which requires both paternal and maternal contributions, mtDNA is inherited solely from the maternal lineage. It is unclear what advantage a uniparental mtDNA transmission confers, but one possibility is to minimize the number of distinct genomes to maximize the efficiency of a multi-genomic system (Hill et al. 2019). In fact, humans have developed complex, redundant mechanisms to ensure uniparental inheritance of mtDNA (DeLuca and O'Farrell 2012; Rojansky et al. 2016). Paternal mitochondria from sperms that enter into the egg during fertilization are actively and selectively eliminated via mitophagy through two E3 ligases, PARKIN, and MUL1 (Rojansky et al. 2016). PARKIN and MUL1 serve redundant purposes, and mitophagy becomes insufficient to eliminate paternal mtDNA only in the absence of both (Rojansky et al. 2016). Even though oocytes have at least a thousand-fold more mitochondria than a sperm cell (Rojansky et al. 2016) and heteroplasmy levels would be very low if paternal mtDNA were to contaminate the embryo, the results can still be non-trivial. However, challenging this notion, a recent study provides evidence of potential paternal transmission (Luo et al. 2018), but awaits further corroborating studies (Lutz-Bonengel and Parson 2019).

MtDNA has a considerable impact on the regulation of nuclear genes (Dunham-Snary et al. 2018; Fetterman and Ballinger 2019; Kopinski et al. 2019; Morava et al. 2019; Mossman et al. 2019; Mottis et al. 2019; Quirós et al. 2016). MtDNA diversity is thought to influence the penetrance and phenotypic expression of pathogenic genetic variants, even within a given family (Morava et al. 2019). For example, a homozygous mutation (c.523delC) in the adenine nucleotide translocator 1 gene (SLC25A4, ANT1) can lead to cardiomyopathy with variable pathological degrees depending on the mtDNA lineage (McManus et al. 2019). Mitochondrial genotype also influences metabolic and epigenomic processes, thereby may underlie phenotypic variability of diseases (Kopinski et al. 2019). Further, mice with artificially matched mitonuclear genomes can exhibit altered physiology, including fertility, metabolism, and gene expression (Dobler et al. 2018). Based on these studies, compatibility between the mitochondrial and nuclear genomes is a key determining factor in organismal fitness.

On this line, mitochondrial replacement therapy (MRT) is a specific form of human gene editing where a mother with known pathological mtDNA can replace her mitochondria with that from another woman. Thus, the baby will have three biological parents that each contributed half of the nuclear genome or the entire mitochondrial genome, often referred to as a "three-parent baby". Combining these genomes would introduce novel mitonuclear combinations that have not undergone natural selection and may increase the risk of developing diseases, especially with age (DeLuca and O'Farrell 2012; Dobler et al. 2018; Hill et al. 2019; Reinhardt et al. 2013). In flies, artificial disruption of mitonuclear epistasis, by generating mutations in the mitochondrial $tRNA^{tyr}$ and its nuclear-encoded mitochondrial tyrosine synthetase, resulted in decreased oxygen consumption, higher mtDNA copy number, higher hydrogen peroxide production, and aggravated age-dependent mitochondrial

dysfunction (Pichaud et al. 2019). Notably, humanin has pleiotropic effects on mtDNA copy number (Kariya et al. 2003; Sreekumar et al. 2016), suggesting a dynamic regulatory role in mitochondrial function and cellular health (Clay Montier et al. 2009; Fazzini et al. 2018). In mice, cross-pairing mitonuclear genomes derived from different strains [mitochondrial nuclear exchange (MNX)] shifts cellular metabolism, oxidative stress levels, resistance to cardiac damage, and atherogenic diet (Betancourt et al. 2014; Dunham-Snary and Ballinger 2015; Fetterman et al. 2013). Mitonuclear interactions associated with components of the MRC can influence function and aging itself in a sex-dependent manner (Immonen et al. 2016). Mitonuclear genomic compatibility may clinically manifest at different stages of life and have a considerable impact on aging and age-related disease.

MtDNA exhibit a higher mutation rate than nuclear DNA, leading to significant population-level mtDNA polymorphisms (van Oven and Kayser 2009; Wallace 1999; Wallace and Chalkia 2013). In fact, the co-evolution of the mitonuclear genomes has been proposed to be driven by mtDNA mutations that select for compensatory changes in the nuclear genome (Havird and Sloan 2016). Populations that share similar mtDNA polymorphisms can be clustered into distinct haplogroups that are designated using all letters of the alphabet (i.e., A through Z). The mtDNA haplogroups represent major branch points on the mitochondrial phylogenetic tree that have strong regional ties around the globe, thus supporting the concept of a ‘mitochondrial eve’ (Wallace 1999). Haplogroups present inherently different mitonuclear interactions (Zaidi and Makova 2019), which eventually affect the aging process (Wolff et al. 2016). For example, one haplogroup commonly found in Ashkenazi Jews can interact with a specific enrichment of an amino acid sequence in complex I, and result in altered susceptibility to type 2 diabetes mellitus (Gershoni et al. 2014). The effect of mitonuclear compatibility on lifespan is influenced by environmental cues in flies (Drummond et al. 2019). It is unclear if mitonuclear compatibility is invariable throughout an organism’s life, or antagonistically pleiotropic during aging, making it a difficult moving target to understand.

NUMTs

The original genome of the endosymbiotic bacteria has been considered to be lost or transferred to the nuclear genome, leading to the current abridged mtDNA (Johnston and Williams 2016). Proto-mitochondrial DNA sequences that have laterally transferred to the nuclear genome are known as NUMTs (Nuclear Mitochondrial DNA segment) (Lopez et al. 1994; Timmis et al. 2004). Further, long and short stretches of the mitochondrial genome are found to be copied into the nuclear DNA, albeit the sequences being degenerate. While the full comprehension of the number of NUMTs in eukaryotes is unknown, current sequencing technology is sufficient to understand NUMT evolution and comparative analyses across species. Interestingly, one study used phylogenetic analysis of NUMTs to show that primates had a greater occurrence of NUMTs than non-primates, and that the clusterizations of these primate NUMTs were intermingled, while non-primate NUMTs were separated by species (Calabrese et al. 2017). Given the relative mutation rates of mitochondrial vs nuclear DNA, NUMTs serve as a “molecular fossil”, and can be used to estimate the time of integration (Perna and Kocher 1996). While there are certain periods of rapid NUMT integration, insertion appears to have been continuous over time leading to the current human genome

(Bensasson et al. 2003; Calabrese et al. 2017; Hazkani-Covo et al. 2010). Notably, mtDNA sequences are still continuously being integrated into the nuclear genome (Ju et al. 2015; Ricchetti et al. 2004; Srinivasainagendra et al. 2017).

The integration of NUMTs into the nuclear genome can lead to problems. While most NUMTs are benign polymorphisms, there are a small number of human diseases associated with NUMTs. The majority of these cases involve the insertion of the NUMT into a nuclear-encoded gene that disrupts proper function (Ahmed et al. 2002; Goldin et al. 2004; Turner et al. 2003). In each of these cases, the nuclear genome is compromised while the mtDNA is intact. Discovering these diseases pose additional challenges. Since NUMTs are of mitochondrial origin, it is difficult to discern mtDNA from nuclear DNA in common methods. When identifying mutations, it becomes easy to confuse nuclear mutations for the much more volatile mitochondrial mutations (Hazkani-Covo et al. 2010). Beside these insertion diseases, there is growing evidence involving NUMTs in cancer (Singh et al. 2017). In one of the first reports on this issue, NUMTs that were nearly the size of the entire mitochondria genome were found in cancer cells (Ju et al. 2015). Another study found that colorectal tumor DNA had roughly four times the number of NUMTs compared to DNA taken from blood cells in the same individual (Srinivasainagendra et al. 2017). Given the emerging role of NUMTs in human diseases including cancer, combined with the increasing ease of sequencing, further findings on the role of NUMTs in disease and evolution are likely around the corner.

Understanding the effects of NUMTs in human pathology involves understanding the mechanisms of their integration into the nuclear genome. This process involves mtDNA exiting the mitochondria, entering the nucleus, and recombination into the nuclear genome. While there is debate as to the frequency of NUMT integration, the frequency of mtDNA transfer to the nucleus is estimated to be 2×10^{-5} per cell per generation (Thorsness and Fox 1990). Furthermore, integration frequency may be that one cell in every 1000–10,000 yeast cells may harbor a new mitochondrial insertion. NUMTs found in the human nuclear genome contain large fragments of non-coding regions of the mtDNA (Huang et al. 2005). This data indicates that it is not cDNA or transcripts that integrate into the nuclear genome, but rather large unedited portions of mtDNA. NUMTs in humans are integrated into the genome through double-strand breaks (DSBs), combined via non-homologous end joining (NHEJ) (Ricchetti et al. 2004). Interestingly, unlike normal NHEJ events, repair involving NUMTs rarely causes deletions and these deletions are small when they do occur (Hazkani-Covo and Covo 2008). Therefore, there is a trade-off between larger deletions to repair DSBs or utilizing mtDNA in the repair process in the form of NUMTs. Deletions may be catastrophic for cells, and insertion of NUMTs, while implicated in disease, may be preferential to the survival of the cell and organism. The number of NUMTs in the genome is small enough to indicate NUMTs are not utilized significantly to stabilize genomic integrity, but no other type of DNA fragments have been found that heal DSBs in a similar manner (Hazkani-Covo et al. 2010). This offers an intriguing role of NUMTs in evolution beyond the ability to regulate OXPHOS components through concerted mitonuclear communication. However, small mtRNA levels are not associated with NUMT abundance across six vertebrate species, but are rather strongly associated with mtDNA content in a tissue-specific manner within species (Pozzi and Dowling 2019).

mtDNA mutations and aging

One of the major components of mitonuclear communication comes as a direct byproduct of OXPHOS activity. Electrons can leak from the MRC and combine with surrounding oxygen molecules to create free radicals and reactive oxygen species (ROS) (Adam-Vizi 2005; Boveris and Chance 1973). These molecules can damage cell components such as protein, lipids, and DNA. Given the high production of ROS in the mitochondria, mtDNA was considered to be particularly susceptible to this damage (Harman 1956). For nearly 50 years, this idea led many to believe that free radicals were largely responsible for mtDNA damage and consequently, a major driver of the aging process. This became known as the mitochondrial free radical theory of aging (MFRTA) (Harman 1956, 2009). However, the effect of antioxidants on longevity has largely been inconclusive (Pomatto and Davies 2018). Only a handful of studies that inactivated various antioxidant systems in model organisms shortened lifespan. These include *sod1* and *sod2* in yeast (Longo et al. 1996; Unlu and Koc 2007), various sod isoforms in worms (Doonan et al. 2008), *sod1* and *sod2* in flies (Martin et al. 2009; Wicks et al. 2009), and *sod1* in mice (Zhang et al. 2017). Conversely, the overexpression of these same genes can increase lifespan in these species (Fabrizio et al. 2003; Melov et al. 2000; Zhang et al. 2016). MFRTA was further reinforced by the fact that mitochondrial repair mechanisms were inferior to their nuclear counterparts, making mtDNA more vulnerable to ROS-induced DNA mutations (Yakes and Van Houten 1997). ROS causes base modifications (hydroxylation) that are effectively fixed by base excision repair (BER) mechanisms. Unlike previously thought, mitochondria are proficient in BER and can effectively repair oxidative mtDNA lesions (Bohr et al. 2002). Further, mtDNA quality is controlled and maintained through numerous mechanisms including mitochondrial fission and fusion (Chen et al. 2010; Prevost et al. 2018), mitophagy (Pickles et al. 2018), distance from MRCs (Cogliati et al. 2016, 2013; Kopek et al. 2012), and physical shielding the mtDNA through clustering in nucleoids (Lee and Han 2017).

Even with these levels of mtDNA protection, mtDNA mutation frequency increases with age in animal models and humans alike (Cortopassi and Arnheim 1990; Larsson 2010), although the role of mtDNA mutations remains unclear (Khrapko and Vijg 2009; Pohjoismaki et al. 2018; Theurey and Pizzo 2018). However, recent reports have shown that mtDNA point mutations in aged tissues largely arise from replication infidelity (i.e., DNA polymerase errors), rather than ROS-induced damage (Ameur et al. 2011; Kennedy et al. 2013; Vermulst et al. 2007). To test if replicative infidelity causes aging, mice with mutant mitochondrial DNA polymerase γ that are deficient in proofreading during DNA replication, causing supraphysiological mutation loads (roughly 2500-fold in the homozygous $polg^{mut/mut}$ compared to 500-fold higher in the $polg^{+/mut}$), were examined (Vermulst et al. 2007). While the homozygous mice ($polg^{mut/mut}$) showed signs of accelerated aging phenotypes and significantly reduced lifespan, the heterozygous mice ($polg^{+/mut}$) had a normal lifespan albeit exhibiting premature aging phenotypes (Trifunovic et al. 2004). One plausible explanation for this discrepancy lies with increased mtDNA deletions in the homozygous mice ($polg^{mut/mut}$) (Vermulst et al. 2007, 2008). These cumulative results suggest that the connections between oxidative stress, mtDNA mutations, and aging are more complicated than originally appreciated and require further investigation to fully understand their relation

(Pomatto and Davies 2018). It is evident, however, that the mtDNA mutations are linked to more than 300 diseases connected to aging, including Alzheimer's Disease, and that proper communication between the mitochondria and the nucleus plays a key role (DeBalsi et al. 2017; Grazina et al. 2006; Lane 2011; Onyango et al. 2006; Quirós et al. 2016; Swerdlow et al. 2017).

Mitonuclear gene regulation

Human cells are based on a bi-genomic system that compartmentalizes each genome in the nucleus and mitochondria. Historically, the nuclear genome was considered to encode for regulators of gene expression for both mitonuclear genomes, whereas mtDNA exclusively encoded for respiratory machinery subunits. However, we recently reported that the mitochondrial-encoded MOTS-c peptide can translocate to the nucleus and directly regulate adaptive nuclear gene expression in response to metabolic stress (Kim et al. 2018a; Mangalhara and Shadel 2018; Wong 2018; Yong and Tang 2018). The stress-induced nuclear translocation of MOTS-c occurred rapidly (< 30 min) and dynamically and required the co-activation of AMPK. MOTS-c can bind DNA and interact with major stress-responsive transcription factors, including Nrf2 and ATF1. A broad range of genes were regulated by MOTS-c under glucose restricted conditions, especially including those related to interferon pathways. Ultimately, the overexpression MOTS-c increased its nuclear presence and significantly protected HEK293 cells from glucose and serum starvation. This study suggests the existence of additional mitochondrial-encoded regulators of nuclear gene expression, where MDPs are prime candidates, especially considering that the mitonuclear genomes co-evolved for over 1.5 billion years as a unified and integrated genetic system.

Mitochondria can also communicate to the nucleus using metabolic intermediates, largely products of the Krebs cycle that serve as substrates for key regulators of nuclear gene expression. Acetyl-CoA is produced by pyruvate dehydrogenase (PDH), a complex normally residing in the mitochondria (Menzies et al. 2016). PDH can also translocate to the nucleus and produce acetyl-CoA in situ. Acetyl-CoA levels are higher in the nucleus and cytosol under growth conditions, where it is used for histone acetylation and lipid synthesis. Conversely, under low-nutrient conditions, mitochondrial acetyl-CoA levels increase to drive ATP production (Shi and Tu 2015; Sutendra et al. 2014). Other metabolites serve similar functions in regulating genetic and epigenetic reprogramming, including oxaloacetate, fumarate, α-ketoglutarate, and malate (Benayoun et al. 2015). NAD^+ is another mitochondrial metabolite involved in mitonuclear communication through its central role in ATP production (Karpac and Jasper 2013; Mouchiroud et al. 2013). Reduced NAD^+ activity is related to lower levels of deacetylase sirtuin activity, which impacts communication between the nucleus and mitochondria (Imai and Guarente 2016). Additionally, NAD^+ levels decline with age, and the resulting decrease in mitonuclear communication results in reduced longevity (Mouchiroud et al. 2013; Yoshino et al. 2011).

Mitochondrial ATP and ROS levels also act as signaling molecules that relay metabolic cues to the nucleus. Reduced ATP synthesis can stimulate AMPK, which in turn activates PGC1α, which then serves to increase mitochondrial energy metabolism and biogenesis (Garcia-Roves et al. 2008; Quirós et al. 2016). Activation of the AMPK pathway also

induces the mitochondrial quality control system and mitophagy (Egan et al. 2011). ROS levels act as a surrogate gauge of mitochondrial respiration activity and efficiency (Murphy 2009). While ROS are often associated with macromolecule damage at higher concentrations, they are key signaling molecules under physiological levels (Sena and Chandel 2012). For instance, antioxidant supplementation can reduce organismal fitness and lifespan by inducing an adaptive stress response (Ristow and Schmeisser 2014, 2011) and dampen skeletal muscle adaptation to exercise training (Merry and Ristow 2015). Also, a mild increase in ROS production delays the aging process in worms (Schulz et al. 2007) and mice (Ristow and Schmeisser 2011), in part, through the activation of array genes that regulate cellular homeostasis under stress (Shadel and Horvath 2015).

MtDNA variation can influence the expression and progression of nuclear DNA mutations (McManus et al. 2019). In this study, researchers knocked out the adenine nucleotide translocator 1 (ANT1) in mice. They found that $ANT1^{-/-}$ resulted in decreased OXPHOS complex I amount, as well as complex V assembly. Additionally, these knockout mice showed that mtDNA mutations enhance the deleterious impact of communication between the mitochondrial and nuclear genomes (McManus et al. 2019). The adverse effects include impaired complex I activity, increased ROS damage, altered mitochondrial morphology, changes to the mitochondrial permeability transition pore, increased mtDNA mutation, and shortened lifespan. Overall, researchers discovered the crucial role that mtDNA variants play in autosomal diseases (McManus et al. 2019).

Multiple studies have linked mtDNA heteroplasmy to nuclear epigenomic changes (Bellizzi et al. 2012; Dunham-Snary et al. 2018; Kopinski et al. 2019; Lee et al. 2017), highlighting the importance of heteroplasmy in proper communication between the genomes. For instance, using cells of the same nuclear background, a mitochondrial genome with increasing levels of the pathogenic mutation ($tRNA^{Leu(UUR)}$ 3243A > G) can be introduced to achieve a gradient of heteroplasmy ranging from 0 to 100% (Kopinski et al. 2019). Interestingly, different levels of heteroplasmy had various effects on nuclear gene expression. Under conditions of high heteroplasmy, the amount of acetyl-CoA decreased, indicative of decreased acetylation of histone H4. Samples with 30–70% of the A3243G heteroplasmy had higher levels of αKG/succinate, which is linked to reduced histone 3 methylation (Kopinski et al. 2019). Additionally, between heteroplasmy levels of 60–70%, the ratio of NAD^+/NADH is elevated, indicating an increase in OXPHOS genes, possibly as a countermeasure to respond to declining mitochondrial function (Fetterman and Ballinger 2019; Kopinski et al. 2019). This finding directly links mtDNA polymorphism to nuclear gene expression.

The mitochondrial unfolded protein response (UPR^{mt}) is an adaptive transcriptional response to mitochondrial stress that promotes cellular homeostasis. Initially, UPR^{mt} was described in mammalian cells and referred to the selective induction of nuclear-encoded genes involved in stress response to mtDNA depletion (Martinus et al. 1996) or accumulation of misfolded proteins in the mitochondrial matrix (Abbott and Turcotte 2014). More recently, G-Protein Pathway Suppressor 2 (GPS2) has been shown to be involved in mitonuclear communication in mammals, regulating insulin signaling, lipid metabolism, and inflammation (Cardamone et al. 2012; Cederquist et al. 2017; Jakobsson et al. 2009). GPS2

translocates to the nucleus upon mitochondrial perturbation and directly activates nuclear-encoded mitochondrial genes, including mitochondrial biogenesis, particularly in brown adipose tissue (Cardamone et al. 2018). In *C. elegans*, the activating transcription factor associated with stress 1 (ATFS-1) is a key mediator of UPR^{mt} (Amrita et al. 2015; Nargund et al. 2012). Under normal conditions, ATFS-1 enters the mitochondria and is degraded through proteolysis. However, under stress conditions, ATFS-1 translocates to the nucleus where it upregulates a number of stress response genes, and also plays a role in chromatin remodeling to promote longevity (Nargund et al. 2012). Notably, ATF5, a mammalian homolog of ATFS-1, also induces mitochondrial proteostasis gene transcription (Fiorese et al. 2016; Tian et al. 2016). In addition, UPR^{mt} can induce chromatin remodeling by specific histone modifications; H3K9 methylation by the histone methyltransferase MET-2 and the nuclear co-factor LIN-65 (Tian et al. 2016) and H3K27 demethylation by histone demethylases (*jmjd-1.2* and *jmjd-3.1*) (Merkwirth et al. 2016).

UPR^{mt} is currently used more inclusively and can refer to adaptive nuclear responses to various types of mitochondrial perturbations, including nutrient availability, iron–sulfur cluster assembly, immune response, and dysfunctional metabolism (Nargund et al. 2015; Shpilka and Haynes 2018; Tauffenberger et al. 2016; Zhu et al. 2014). Notably, whereas UPR^{mt} recognizes the loss of mitochondrial proteostasis, the release of bacterial-like mitochondrial components, including formylated proteins and mtDNA, can act as damage-associated molecular patterns (DAMPs) and trigger an immune response (Grazioli and Pugin 2018; Wenceslau et al. 2014; Zhang et al. 2010). Notably, mtDNA levels in circulation increase with stress and age and are associated with higher levels of inflammatory markers (Pinti et al. 2014; Trumpff et al. 2019). In skeletal muscle, which is metabolically highly active, silencing of miRNA-382 results in UPR^{mt} activation through an imbalance in mitonuclear proteins, induction of HSP60, and downregulation of mitochondrial ribosomal proteins (Dahlmans et al. 2019). Further, nicotinamide mononucleotide (NMN) treatment prevents mitonuclear protein imbalance in mouse muscles (Mills et al. 2016). UPR^{mt} activation in worms, by genetic perturbation of mitochondrial ribosomal protein S5 (*MRPS5*) or pharmacological treatment (ethidium bromide, rapamycin, and resveratrol), extended lifespan. (Houtkooper et al. 2013). Further, mitochondrial stress increases the expression and mitochondrial localization of androgen receptor (AR), which then regulates nuclear-encoded mitochondrial ribosomal proteins and the mitochondrial translation machinery, indicating an adaptive mitonuclear cooperation (Bajpai et al. 2019). Collectively, these findings, and many others, highlight the tight-knit cellular system balancing nuclear and mitochondrial proteins coordinated through UPR^{mt}.

Conclusion

Our genomic system is comprised of both mitochondrial and nuclear genes. Mounting evidence indicates that a highly integrated cross-organellar regulatory mechanism, and overall genomic compatibility, is key to adaptive gene expression and cellular fitness. Mitochondria-to-nucleus communication is a dynamic and inclusive process that reflects many aspects of mitochondrial biology, perhaps to provide the nucleus with an accurate cellular context for adaptive gene expression. A variety of molecular mediators allow close communication between mitochondria and the nucleus, including mitochondrial-encoded

factors that can directly regulate the nuclear genome, metabolic intermediates, ROS, UPR^{mt}, and overall mitonuclear genomic compatibility. Given the uncertainty of mtDNA mutation accumulation in driving the natural aging process, it is plausible that mitochondrial communication may be a significant evolutionarily conserved force that influences lifespan and/or healthspan.

Acknowledgements

Funding was provided by the American Federation for Aging Research (AFAR), the National Institute on Aging (T32 training grant AG052374, AG052558) and the USC Manning Endowed Fellowship to J.C.R., an American Association of University Women (AAUW) fellowship to C.P.B., and the NIA (R01AG052258), Ellison Medical Foundation (EMF), AFAR, and the Hanson-Thorell Family to C.L.

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Table 1

Effects of MOTS-c in various cellular processes

	Effects of MOTS-c	Models	References
Metabolism	Targets the methionine-folate cycle, increases AICAR levels, and activates AMPK	In vitro	Lee et al. (2015)
	Regulates cellular glucose, mitochondrial, and fatty acid metabolism		
	Targets skeletal muscles and regulates insulin sensitivity	Mice	Lee et al. (2015)
	Prevents high-fat diet-induced obesity and insulin resistance		
	Reduces fat mass and improved OVX-induced lipid deposition in the liver	Mice	Lu et al. (2019b)
	Reduces adipocyte size and suppresses adipose-inflammatory response, enhances lipid catabolism, and activates the brown adipose, in OVX mice		
	Circulating levels of MOTS-c is decreased in T2D	Human	Ramanjaneya et al. (2019a)
	Lean and obese people have similar plasma MOTS-c concentrations, but MOTS-c levels are associated with insulin sensitivity in lean, but not in obese people	Human	Cataldo et al. (2018)
	Plasma MOTS-c decreases in obese male children and adolescents and decreases more significantly when they are already obese and insulin resistant	Human	Du et al. (2018)
	Plasma MOTS-c negatively correlates with body mass index, waist circumference, and fasting insulin in male obese children and adolescents		
	Low endogenous plasma MOTS-c is associated with impaired coronary endothelial function (human), and MOTS-c treatment improves endothelial function in rodents	Human Rodent	Qin et al. (2018a)
	MOTS-c treatment regulates plasma metabolites, reduces fat accumulation in muscle, improves insulin sensitivity in diet-induced obese mice	Mice	Kim et al. (2019b)
	MOTS-c treatment improves metabolic status and dermal aging in D-gal-induced aging mice and alleviates lipid accumulation in liver	Mice	Li et al. (2019)
	Lipid enhances circulating MOTS-c while insulin attenuates the MOTS-c response in human	Human	Ramanjaneya et al. (2019b)
	Circulating and skeletal muscle MOTS-c levels are decreased in chronic kidney disease patients	Human	Liu et al. (2019)
Bone biology	MOTS-c treatment alleviates bone erosion by inhibiting osteoclastogenesis through the regulation of osteocyte OPG/RANKL secretion in an ultra-high molecular weight polyethylene (UHMWPE) particle-induced osteolysis mouse model	Mice	Yan et al. (2019)
	MOTS-c treatment alleviates bone loss and inhibits (RANKL) osteoclast differentiation	Mice	Ming et al. (2016)
Gene variants	K14Q-MOTS-c is specific for the Northeast Asian population who are known to have long lifespan	Human	Fuku et al. (2015)
	K14Q-MOTS-c is associated with type 2 diabetes with lower MVPA in men, but not in women	Human	Zempo et al. (2016)
	K14Q-MOTS-c affects glucose tolerance in male mice. These suggest that K14Q-MOTS-c by m.1382 A > C polymorphism may influence prevalence of type 2 diabetes		
	Males, but not females, with K14Q-MOTS-c exhibit higher prevalence of T2D	Human Mice	Zempo et al. (2019)
	K14Q-MOTS-c has reduced insulin sensitization effects compared to MOTS-c, and is less effective in reducing the body weight, fat mass, and glucose tolerance in CD-1 male mice exposed to high fat diet		

	Effects of MOTS-c	Models	References
Senescence	MOTS-c is increased in senescent primary human fibroblasts, and MOTS-c treatment increases mitochondrial respiration and selected components of the SASPs in doxorubicin-induced senescent cells partially via the JAK signaling pathway	Human	Kim et al. (2018b)
Immunity	MOTS-c improves the survival in mice with MRSA infection and enhances bactericidal function of macrophages	Mice	Zhai et al. (2017)
	MOTS-c has anti-inflammatory effects in macrophages stimulated with MRSA	Mice	Zhai et al. (2017)
	MOTS-c treatment in ultra-high molecular weight polyethylene particle-induced osteolysis mouse model alleviates inflammation by restraining NF-κB and STAT1 pathway	Mice	Yan et al. (2019)
	There is a decrease in MDP-coding genes *MT-RNR1* (MOTS-c) expression in chronic fatigue syndrome (CFS), Q fever fatigue syndrome (QFS), and, to a lesser extent, in Q fever seropositive controls	Human	Raijmakers et al. (2019)
Adaptive stress response	MOTS-c translocates to the nucleus to regulate the adaptive nuclear genome expression in response to metabolic stress	In vitro	Kim et al. (2018a)
	MOTS-c alleviates mitochondrial dysfunction caused by $PM_{2.5}$ nanoparticle exposure and higher methylation in *MT-RNR1* of the mtDNA D-loop is associated with higher MOTS-c level suggesting that MOTS-c may be regulated partially by mtDNA methylation in humans	In vitro	Breton et al. (2019)
	MOTS-c treatment promotes cold adaptation, decreases lipid accumulation upon acute cold exposure, and increases the white fat browning and brown fat activation upon acute cold exposure in mice	Mice	Lu et al. (2019a)
Signaling pathway	MOTS-c functions that are dependent on AMPK activity	Misc	Kim et al. (2018a; Lee et al. (2015); Lu et al. (2019b); Ming et al. (2016); Yan et al. (2019)