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Mitochondrial genome maintenance in health and disease

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Abstract

Human mitochondria harbor an essential, high copy number, 16,569 base pair, circular DNA genome that encodes 13 gene products required for electron transport and oxidative phosphorylation. Mutation of this genome can compromise cellular respiration, ultimately resulting in a variety of progressive metabolic diseases collectively known as ‘mitochondrial diseases’. Mutagenesis of mtDNA and the persistence of mtDNA mutations in cells and tissues is a complex topic, involving the interplay of DNA replication, DNA damage and repair, purifying selection, organelle dynamics, mitophagy, and aging. We briefly review these general elements that affect maintenance of mtDNA, and we focus on nuclear genes encoding the mtDNA replication machinery that can perturb the genetic integrity of the mitochondrial genome.

Keywords

POLG; Mitochondrial DNA replication; mtDNA; Mitochondrial disease; Mutagenesis

1. Introduction

1.1. Pol γ mtDNA replication

Of the 17 eukaryotic DNA polymerases, pol γ is the only replicative DNA polymerase known to function within mitochondria [1–5]. The trimeric holoenzyme of this essential polymerase consists of one catalytic subunit (encoded by *POLG* at chromosomal locus 15q25) and a dimeric form of its accessory subunit (encoded by *POLG2* at chromosomal locus 17q24.1). The 140 kDa catalytic subunit (p140) possesses DNA polymerase, 3′-5′ exonuclease and 5′ dRP lyase activities [6], and the 55 kDa accessory subunit (p55) is required for tight DNA binding and fast, processive DNA synthesis [7,8]. The pol γ holoenzyme functions in conjunction with the mitochondrial DNA helicase, Twinkle, and the mtSSB to form a minimal replication apparatus in vitro [9] (Fig. 1A). Recently, the *MGME1* locus (20p11.23) was shown to be mutated in mitochondrial disease patients exhibiting mtDNA depletion and deletions. The *MGME1* enzyme is a RecB-type 5′-3′

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

exonuclease of the PD-(D/E)XK nuclease superfamily and is postulated to have direct involvement in maintenance of mtDNA and turnover of prematurely terminated 7S mtDNA replication intermediates [10,11].

1.2. Sources of mutations in mtDNA

Mutations in mtDNA can arise through spontaneous errors of DNA replication or through unrepaired damage to mtDNA that introduces mis-coding lesions. Due to high nucleotide selectivity and exonucleolytic proofreading, the isolated catalytic subunit of pol γ exhibits exceptionally high fidelity of DNA replication, with nucleotide misinsertion events occurring only once per 500,000 nucleotides synthesized [12]. The intrinsic 3' to 5' exonuclease activity that contributes to replication fidelity can be completely eliminated by substitution of alanine for Asp198 and Glu200 in the conserved ExoI motif of human pol γ [13]. Comparing the *in vitro* error rates for the exonuclease proficient and deficient forms of pol γ indicates that exonucleolytic proofreading contributes at least 20-fold to the fidelity of mtDNA synthesis [12]. Inclusion of the p55 accessory subunit decreases both frameshift and base substitution fidelity. Kinetic analyses indicate that p55 lowers fidelity of replication by promoting extension of mismatched DNA termini [12]. Nevertheless, the spectrum of base substitution errors made by highly purified pol γ copying DNA *in vitro* has been measured, and the resulting mutations represent over 85% of the mutations detected in native mtDNA that has been maintained *in vivo* [14]. This result is remarkable, because mutations in native mtDNA represent the net sum of replication errors, unrepaired chemical damage to mtDNA, and purifying selection over many cell generations. Thus, spontaneous replication errors by pol γ account for the majority of base substitution mutations in mtDNA. By extension, spontaneous errors by pol γ are most likely responsible for the accumulation of point mutations and deletions in mtDNA during aging [15–18]. Ultra sensitive sequencing has determined that the frequency of point mutations increases approximately 5-fold over the course of 80 years of life [19]. These mutations are predominantly transition mutations, which is consistent with their proposed origin as common Pol γ mediated misincorporation events. Interestingly, G to T transversion mutations that are commonly associated with oxidative damage (generated from reactive oxygen species as a by-product of the electron transport chain) do not significantly increase with age, suggesting that oxidative damage to mtDNA may not be a significant factor in aging [19].

MtDNA mutations in cancer cells have been suggested to contribute to the development of cancer [20]. However, the notion of a causal role for mtDNA mutations was challenged by a recent analysis of colorectal tumor tissue that showed a decrease mtDNA mutagenesis as compared to adjacent normal tissue [21]. The major reduction of mutations was due to a decrease in C:G to T:A transitions, which are associated with oxidative damage or Pol γ biosynthetic errors. Tumor cells are more reliant on glycolysis for energy production than normal cells, and this 'Warburg Effect' depresses mitochondrial respiration. Reduced respiration lowers mitochondrial biogenesis and attendant DNA replication errors. Taken together, decreased mitochondrial biogenesis and lowered oxidative damage result in reduced mutagenesis.

1.3. Pol γ exonuclease activity, disease, and mtDNA mutagenesis

To test the potential effects of eliminating mitochondrial proofreading function on disease, several groups have employed mouse models with disrupted pol γ exonuclease activity. In the first mouse model, a transgenic pol γ variant that eliminated pol γ exonuclease activity was targeted to the heart, where it caused severe cardiomyopathy accompanied by mtDNA mutations and deletions [22]. Several years later, two independent groups created knock-in mice homozygous for mutations that disrupted exonuclease function [23,24]. These mice exhibited premature aging between six and nine months, characterized by graying hair, loss of hair and hearing, curvature of the spine, enlarged hearts, and decreased body weight and bone density [23,24]. These observations have not only shown that exonuclease-deficiency in pol γ does not cause embryonic lethality, but they also have stimulated discussion about the role of mtDNA mutations in aging and mitochondrial disease.

The degree of increased mtDNA mutagenesis in *POLG* exonuclease deficient mice was originally unclear. The increase in mtDNA mutagenesis reported in the mutant mice (three- to eight-fold increase) is similar to the accumulation of mutations detected in two- to three-year-old wild-type mice (three- to eleven-fold) [23–26]. However, mutation frequencies in young, wild-type mice is at or below the limit of detection using methods based on PCR cloning and sequencing, which introduces mutations at 1.3×10^{-4} mutations per base pair [24]. This limitation was alleviated by an alternative method of quantifying mutation frequencies called the “random capture method,” where the frequency of mutations that cause resistance to restriction endonuclease digestion is enriched, allowing more accurate estimations of mutation frequency (7.1×10^{-7} mutations per base pair in wild-type mice and 1.6×10^{-4} mutations per base pair in young heterozygotes) [25]. Mutation frequency in homozygous mutant mice was confirmed using next-generation sequencing technology [27]. The mutation frequency of heterozygotes, which were asymptomatic, was much higher than aged wild-type mice (5.4×10^{-6} mutations per base pair) [25]. Therefore, the increase in mutation frequency that occurs in older mice is not sufficient to cause phenotypes associated with aging. However, it is still possible that the extremely high mutation rate that occurs in homozygous *POLG* exonuclease-deficient mice is sufficient to promote or accelerate the aging process.

In addition to detecting point mutations, the random capture assay detected a 90-fold increase in mtDNA deletions in homozygous *POLG* exonuclease-deficient mice as compared to age-matched wild-type or heterozygotes [28]. While mtDNA deletions in wild-type and heterozygotes mostly occur between direct repeats of six or more nucleotides, mtDNA deletions in homozygous mutants occur independently of direct repeats [28]. The mechanism for deletions between direct repeats is often attributed to strand-slippage, where a mispriming event occurs downstream of the correct target, a process that appears to be significantly dampened by the proofreading exonuclease function of a polymerase. Interestingly, the lack of increase of deletions in the heterozygote suggests that the wild-type copy of pol γ is able to protect against deletions that are caused by the exonuclease-deficient variant, suggesting an interplay between separate domains of both enzymes similar to the idea of *extrinsic* proofreading [29]. The role of the exonuclease in the formation of mtDNA deletions between direct repeat was tested in yeast and shown to suppress the formation of

deletions [30]. The role of mtDNA deletions in aging is controversial because there is uncertainty about the absolute number of mtDNA deletions in the mitochondria. Because the random capture assay only detects relative numbers of deletions among various samples, deletion quantification has depended on southern blots and long-range PCR techniques that are helpful when the majority of molecules have a particular deletion [31], but not when the size and distribution of deletions are expected to be random [32]. Next-generation sequencing techniques detected the presence of deletions or tandem duplications only in the control region (the technique cannot distinguish between the two) in homozygous mutant mice [27]. Other studies have found relatively abundant levels of linear non-replicating molecules with large mtDNA deletions, probably due to pausing at the control and OriL regions during mtDNA replication [33,34]. However, amplification of large sections of single mtDNA molecules extracted from two- to three-year-old mice showed that only 0.07–0.20% of molecules contained mtDNA deletions, arguing against the model that mtDNA deletions drive normal aging [35]. Similar to the story of mtDNA mutations, mtDNA deletions may not contribute to normal aging, and their role in premature aging in mice with exonuclease-deficient pol γ remains unclear.

2. Pol γ and mtDNA replication proteins in disease

Mitochondrial diseases can be caused by genetic defects in mtDNA or in nuclear genes that encode proteins that function in the mitochondria [36]. Studies over the last decade have identified a list of genes linked to instability of mtDNA, either due to mtDNA depletion syndrome (MDS) or disorders characterized by multiple deletions, with or without point mutations. The current list of genes associated with MDS and deletion syndromes is presented in Table 1 [37,38]. Over 200 mutations in *POLG* are associated with various mitochondrial diseases (<http://tools.niehs.nih.gov/polg/>) [37,39–43]. *POLG*-related disorders are currently defined by at least five major phenotypes of neurodegenerative disease (Table 2) that include: (1) Alpers-Huttenlocher syndrome (AHS), (2) childhood myocerebrohepatopathy spectrum (MCHS), (3) myoclonic epilepsy myopathy sensory ataxia (MEMSA), (4) the ataxia neuropathy spectrum (ANS), and (5) progressive external ophthalmoplegia (PEO) with or without sensory ataxic neuropathy and dysarthria (SANDO) [44–47]. These diseases are associated with mutations that have been shown to decrease enzymatic activities of the polymerase and helicase, to reduce the fidelity of DNA replication, to disrupt formation of the polymerase holoenzyme, and to alter assembly of the replication machinery.

Biochemical and genetic analyses have demonstrated the consequences of disease mutations in *POLG* (reviewed in [42,48,49]). A few mutations are worth noting due to their unusual frequency and/or significance to health as they relate to the structure of the polymerase. The common Y955C amino acid substitution causes autosomal dominant PEO [50,51]. Tyr955 is also located in the active site at the C terminus of the O-helix [52]. Together with Glu895 and Tyr951, Tyr955 forms a hydrophobic pocket to receive the incoming dNTP. Disruption of the hydrogen bonding of Tyr955, Tyr951, and Glu895 decreases both DNA replication fidelity [53] and discrimination against antiviral nucleotide analogs [13,54]. In addition, Tyr955 with Phe961 allows for either error-free bypass or translesion synthesis of 8-oxo-dG in the template DNA strand by keeping 8-oxo-dG in the *anti*-conformation and favoring

binding to dCTP. In contrast, the cysteine substitution at position 955 allows for the *syn*-conformation of 8-oxo-dG which can accommodate pairing with dATP *via* Hoogsteen hydrogen bonding [55]. Because there is an increase in mitochondrial oxidative damage caused by Y955C in yeast and also in transgenic mouse models [56,57], error-prone bypass or misincorporation opposite 8-oxo-dG by Y955 C pol γ may contribute to the detected increase in mitochondrial mutagenesis [58].

The crystal structure of human pol γ holoenzyme was determined to within 3.2 Å, confirming biochemical evidence that the holoenzyme is a heterotrimer comprised of two accessory subunits and one pol γ catalytic subunit [59,60]. The contact between the catalytic subunit and the accessory homodimer is asymmetric with the catalytic subunit primarily contacting the proximal p55 monomer [60]. Each p55 monomer contributes to the overall processivity of the holoenzyme. The distal monomer enhances the polymerization rate, and the proximal monomer increases DNA binding affinity of the holoenzyme [61]. Pol γ p140 amino acids Arg232 and Glu540 comprise the main contact with the distal p55 monomer [60]. Mutations that change Arg232 to glycine and histidine have been identified in several unrelated compound heterozygous patients afflicted with Alpers or Alpers-like infantile hepatocerebral diseases [62–67]. Although mutations at Arg232 do not affect pol γ p140 alone, the mutant holoenzyme displays decreased polymerase activity and exonuclease selectivity for mismatched base pairs [68]. Contact with the proximal subunit is much more substantial, involving hydrophilic interactions of amino acids in the N-terminal thumb domain area and positively charged residues (Lys1198, Arg1208, and Arg1209) in the palm domain [60]. Hydrophobic interactions involving a helix comprised of amino acids 543–558 in the spacer region predominantly stabilizes subunit interaction. In pol γ p140 mutants that disrupt these hydrophobic interactions, the processivity is not enhanced by the accessory subunit. Ala467, which resides in the DNA-interacting thumb domain, is also important for pol γ subunit interaction. The most common disease-causing pol γ mutation is A467T, which causes decreased binding to accessory subunit along with overall decreased polymerase activity [69,70]. The Ala467 is located in the thumb domain which interacts with the accessory subunit and substitution to threonine is thought to interrupt the hydrophobic area formed by nearby leucine residues causing a shift of this region [60].

Mutation W748S is the second most common pol γ disease mutation and associates with Alpers and ataxia [71,72]. W748S causes low polymerase activity and processivity along with defective DNA binding [73]. In patients, W748S is usually associated with the neutral polymorphism E1143G, and the double mutant improves DNA binding and polymerase activity compared to the single W748S. The crystal structure shows that Trp748 associates with other aromatic residues Phe750 and His733 that must be stabilized to bind effectively to template DNA [60]. Trp748 faces away from the interface of the monomer of p55 and has no effect on binding to p55 [60,73].

The side chain substitutions G848S, T851A, R852C, and R853Q are located in a conserved region of the thumb domain, and all are associated with Alpers syndrome [50,66,74,75]. In each case, the mutations were shown to nearly eliminate polymerase activity [76]. In addition, G848S and R852C also showed a four- to five-fold decrease in binding affinity to DNA [76]. Arg853 is located very close to and may provide electrostatic interaction with

one of the magnesium-chelating aspartic acid residues [60]. A change to glutamine from Arg853 would cause a repulsive electrostatic interaction and could explain the dramatic decrease in catalytic activity. Interestingly, R853W has been associated with PEO [77,78], and genetic data of the yeast homolog suggests that polymerase activity is not as severely compromised [79]. The tryptophan substitution is not expected to repel the nearby aspartic acid, but the bulky side chain may cause steric clashes.

Surprisingly, mutations that have been studied in the exonuclease domain, which are most conserved from humans to yeast, have not caused increases in mutagenesis *in vivo* or a decrease in exonuclease activity *in vitro* [58,79,80]. In fact, kinetic data showed that disease-associated mutations increase exonuclease activity for both correct and mismatched primer-template termini [68,80]. Because not all disease-associated mutations have been assayed for exonuclease activity, it is still possible that compromised proofreading function can cause *POLG*-related diseases. However, proofreading activity may not factor into diseases that clinicians identify with *POLG* mutations. It is unknown if exonuclease-deficient pol γ variants are found in the unaffected population or in people affected by noncanonical *POLG*-associated diseases. It is also formally possible that mutations that eliminate exonuclease function of pol γ are embryonic lethal. This seems unlikely considering that mice with exonuclease-deficient pol γ are viable [23,24] and exonuclease-deficient pol γ efficiently replicates DNA *in vitro*.

3. DNA repair pathways in mitochondria

Mitochondrial DNA incurs chemical damage from both endogenous and exogenous sources which can result in mutations. A major source of damage to mtDNA is oxidative damage, which is caused by reactive oxygen species generated through leakage of electrons from the electron transport chain. Mitochondria have certain DNA repair systems to counter such damage. Recently, several comprehensive reviews have been published that describe the types of damage detected in mtDNA and mitochondrial DNA repair pathways [81–83]. For this issue, we felt it was important to present the main mtDNA repair pathways as they compare to nuclear DNA repair pathways and to introduce a unique ‘mitochondrial repair’ system not present in the nucleus.

3.1. Base excision repair (BER)

Mitochondria possess a robust system for base excision repair. BER is initiated by a host of DNA glycosylases, which recognize numerous types of base damage. Mitochondrial base excision repair can proceed via two pathways, single-nucleotide-BER (SN-BER) or long-patch BER (LP-BER) [84] (Fig. 1B). In both repair pathways, an oxidized or damaged base is recognized and cleaved by a specific glycosylase, leaving an abasic site that is cleaved on the 5' end by AP endonuclease to generate a nick with a 5'-deoxyribose phosphate (dRP) flap. During single nucleotide BER, the mitochondrial DNA polymerase, pol γ fills the gap and cleaves the 5'-dRP moiety prior to ligation [85].

LP-BER activity in mitochondrial extracts has been described, and the mitochondrial proteins required for LP-BER have been identified [86–88]. LP-BER requires an activity to remove the displaced 5'-DNA strand, commonly known as a 5'-flap structure, and Liu et al.

found that FEN-1 in their mitochondrial preparations that could carry out this activity *in vitro* [87]. Furthermore, DNA2, originally identified as a yeast nuclear DNA helicase with endonuclease activity, has also been implicated in mitochondrial LP-BER, as well as having a possible role in mtDNA replication [89,90]. In this capacity, DNA2 functions with FEN-1 to process 5' protruding flaps due to strand displacement synthesis during LP-BER prior to ligation by ligase III. Alternately, the 5' end may be processed by EXOG to produce a substrate for ligation [91]. It is presently unclear if the newly identified MGME1 exonuclease can also participate in this type of reaction [10,11].

3.2. Nucleotide excision repair (NER)

It was demonstrated in 1975 that mitochondria cannot repair UV induced pyrimidine dimers and lack a nucleotide excision repair system [92]. Subsequent studies have validated the lack of NER in mitochondria and shown that UV exposure promotes C to T mutations in mtDNA [93]. Alkylation damage to mtDNA has also been investigated, and such lesions were found not to be repaired by NER in mitochondria [94].

3.3. Mismatch repair (MMR)

The presence of mismatch repair (MMR) pathways required for the removal of base mismatches and short insertions and deletions in nuclear DNA is well established. However, there is only limited evidence of mismatch repair machinery in mitochondria. The presence of MMR in mitochondria has been reported in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* [95–97] but not in higher eukaryotes. *S. cerevisiae* encodes msh-1, a homologue of *Escherichia coli* MutS, and mutations in msh-1 induce a higher mutation rate in yeast mtDNA [98,99]. However, no homologue of msh-1 has been found in animal cells. A homologue of bacterial MutS (MSH) gene has been found in the mitochondrial genome of the soft coral [100,101] and suggests a mismatch repair activity in coral mitochondria.

One report has described activities in rat liver mitochondria that resembles MMR [102]. These mitochondrial lysates repaired G•T and G•G mismatches in bidirectional, ATP-dependent reactions, but they did not exhibit a strand bias for correcting these base–base heterologies. The reported activity was much less potent than nuclear MMR, and no MSHs were detected in purified mitochondrial extracts, suggesting that different enzymes/mechanisms may be involved [102]. In a search for proteins responsible for this activity a mismatch specific DNA binding protein, YB-1, was isolated and shown to localize to human mitochondria [103]. Depletion of YB-1 in cultured cells increased mutagenesis of mtDNA, suggesting a role in DNA repair or mutation avoidance. How this protein affects mtDNA mutagenesis and its detailed role in mitochondrial mismatch repair is presently unclear.

3.4. Ribonucleotides in mtDNA and the lack of RER

It was first reported in 1973 that mitochondrial DNA contains approximately 10 ribonucleotides per genome [104]. A more recent analysis revealed that the average mitochondrial genome contains as many as 30 ribonucleotides [105]. The source of these ribonucleotides has been widely debated, with potential sources ranging from residual RNA primers following random initiation of mtDNA replication throughout the genome to

indiscriminate incorporation during DNA synthesis [105]. The strand-coupled model of DNA replication is partially founded on the observation of replication intermediates that contain RNA/DNA heteroduplexes, as well as extensive RNA-rich regions in the lagging strand, which implicates direct incorporation of ribonucleotides during nascent strand DNA synthesis [106].

DNA polymerases discriminate against ribonucleotides with as much as a 10,000-fold preference for deoxyribonucleotides, despite the presence of ribonucleotides at much higher concentrations compared to their deoxyribonucleotide counterparts [107,108]. Efficient discrimination of ribonucleotides by these enzymes is controlled by specific amino acid residues, which sterically block entry of the incoming rNMP into the enzyme's active site. However, the identity of these steric gate side chains varies among different polymerases [109–112]. Goff and colleagues first identified a phenylalanine residue (Phe155) in Moloney murine leukemia viral reverse transcriptase that confers selectivity against ribonucleotides [107]. Phe155 in Moloney murine leukemia viral reverse transcriptase is analogous to *E. coli* pol I Glu710 and to T7 DNA polymerase Glu480. Alteration of Glu710 in *E. coli* pol I to Ala results in an 800-fold decrease in overall discrimination against rNTPs, and hence Glu710 is thought to provide discrimination against rNTPs by sterically blocking the 2'—OH group of an incoming rNTP [109]. The structure of T7 DNA polymerase indicates that Glu480 interacts with the ribose ring of the incoming dNTP as well as through hydrogen bonding to Tyr530 [113]. Glu895 of human DNA pol γ is analogous to *E. coli* pol I Glu710 and T7 DNA polymerase Glu480, and alteration of Glu to Ala results in a 100-fold loss of discrimination against ribonucleotides, but also at a cost of greatly reduced DNA polymerase activity [114]. We found that the human mitochondrial DNA polymerase γ discriminates ribonucleotides efficiently but differentially depending on the identity of the base. While, UTP is discriminated by 77,000-fold compared to dTTP, the discrimination drops to 1100-fold for GTP versus dGTP [114]. Since much of the discrimination is Km mediated, the actual discrimination *in vivo* depends on the concentrations of deoxyribonucleotides relative to ribonucleotides. A recent study has demonstrated that mitochondrial rNTP/dNTP ratios in rat tissue varied depending on the specific nucleotide and tissue. In general, the ATP/dATP ratio is ~1000, UTP/dTTP is 9–73, CTP/dCTP is 6–12, and GTP/dGTP is 2–26 in these rat tissues [115]. Thus, in consideration of the relatively high ATP concentration as well as kinetic discrimination against ATP, pol γ has the theoretical potential to incorporate at least 1 rATP for every 10 template T residues [114].

RNase H enzymes can remove the RNA incorporated into DNA, and this 'repair' process is termed ribonucleotide excision repair (RER) [116,117]. Eukaryotic cells contains two RNase H enzymes, a RNase H1 that functions processively to cleave long stretches of RNA/DNA hybrids and RNase H2 that removes singly incorporated ribonucleoside 5'-monophosphate (rNMP) residues in DNA. Whereas both the H1 and H2 enzymes are found in the nucleus, only RNase H1 is thought to function in mitochondria [118], and mechanisms to remove singly incorporated rNMP residues from mtDNA have not yet been identified. However, single ribonucleotides in the template DNA strand can be easily bypassed during replication, because pol γ possesses a strong reverse transcriptase activity. A more fundamental problem to consider is how mitochondria have evolved to tolerate

rNMP residues in DNA. During electron transport, protons are pumped across the inner mitochondrial membrane into the inter-membrane space, forming an electrochemical gradient that drives ATP synthase (complex V). This electrochemical gradient concomitantly acidifies the IMS and raises the pH of the mitochondrial matrix to pH = 7.7 [119]. MtDNA resides in the mitochondrial matrix within nucleoids that are associated with the inner membrane. Ribonucleotides within mtDNA are presumably more susceptible to base hydrolysis than dNMP residues [104], yet turnover of rNMP residues in mtDNA is poorly understood. Perhaps sequestration of mtDNA into nucleoids exerts a protective effect to ribo-substituted mtDNA.

4. Mitophagy: Purging damaged mitochondria through mitochondrial dynamics

Despite highly accurate DNA synthesis by Pol γ and the clear presence of DNA repair systems for the mitochondrial genome, point mutations and deletions in mitochondrial DNA accumulate with age [15–18]. It has been suggested that mtDNA is more susceptible to damage than nuclear DNA, possibly due to the concentration of lipophilic agents within the organelle [120,121]. In multiple organisms such as yeast, rodents and humans, oxidative stress accounts for up to 10-fold more mtDNA damage than nuclear DNA damage [122–125]. Differential persistence of DNA damage in mtDNA is also a factor, as evidenced by a demonstration that repair of hydrogen peroxide-induced lesions in mtDNA occurs more slowly than repair of nuclear DNA damage [122]. Evolutionary arguments based on RFLP analysis of animals also suggest the frequency of point mutation in mtDNA exceeds that of nuclear DNA by a factor of ten [126].

Animal cells maintain a high copy number of mtDNA, and functional complementation of these genomes serves to delay mitochondrial dysfunction despite the ongoing accumulation of mutations and deletions in mtDNA. The mitochondrial network is highly dynamic, and the contents of the organelles are mixed and exchanged by nearly continuous cycles of mitochondrial fission and fusion. Mitochondrial fusion exerts a protective effect in the face of mtDNA mutagenesis [127], and, in disease models, manipulation of mitochondrial fission and fusion can partially rescue disease phenotypes [128]. Specifically, conditional knockout of the mitofusin GTPases Mfn1 and Mfn2 in a Pol γ -D257A mutator background leads to increased lethality, severe mitochondrial dysfunction, and very high levels of mtDNA mutations and deletions [127]. Eventually, the mutant fraction of mtDNA exceeds the phenotypic threshold, and cellular metabolism can be disrupted by mitochondrial dysfunction. However, the cell also has the ability to purge damaged mtDNA and mitochondria through a specialized autophagy event termed mitophagy [129,130]. Mitophagy selectively degrades dysfunctional mitochondria that lack a membrane potential, and this process also serves to enrich the cellular pool of functional mtDNA.

Other examples of organelle dynamics removing damaged mitochondria have been described which relate to Parkinson's disease and a potential therapy. PINK1 and Parkin are implicated in the quality control network to remove dysfunctional mitochondria *via* mitophagy [129]. Recent RNAi screens have identified four other genes, TOMM7, HSPAILL, BAG4 and SIAH3, that help Parkin to tag damaged mitochondria for degradation

[131]. Research by Tarnopolsky and co-workers has demonstrated the benefits of physical exercise as a therapy to reverse aging and mtDNA mutations in the Pol γ -D257A mutator mouse [132]. The role of mitochondrial biogenesis, dynamics, and mitophagy is an exciting area of current and future research.

5. Conclusion

Research on mitochondrial DNA replication and mutagenesis has revealed the following observations: (1) replication and repair of mtDNA are dependent on the nuclear encoded DNA polymerase γ (Pol γ); (2) the main source of both disease mutations and neutral variants in the mtDNA population are spontaneous replication errors made by Pol γ ; (3) the high copy number of mtDNA facilitates tolerance of random point mutations that have minimal effect on mitochondrial function; (4) mitochondrial disease can result from depletion of mtDNA or accumulation of deletions or near homoplasmic point mutations in mtDNA; (5) accumulation of point mutations in mtDNA is associated with aging; (6) oxidative damage to DNA is not a major source of age-related mtDNA mutations; (7) although mitochondria have efficient base excision repair systems, they lack effective mismatch repair and completely lack nucleotide and ribonucleotide excision repair systems; (8) mitophagy and mitochondrial dynamics play essential roles in purging dysfunctional mitochondria. All these processes are the subject of active research to decipher how the cell maximizes mitochondrial function in the face of endogenous and environmental insults in human health.

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A. Proteins at the mtDNA replication fork

B. MtDNA Base Excision Repair

Fig. 1.

Cartoon depicting the major proteins involved in replication and repair of the mitochondrial genome. (A) Proteins involved in strand displacement synthesis. (B) Single nucleotide and long patch base excision repair pathways.

Table 1

Nuclear genes identified in mitochondrial disease patients that affect the stability of mitochondrial DNA.

Gene	Disorder	Chromosomal locus	Function
<i>POLG</i>	PEO/Alpers/ataxia	15q25	Mitochondrial DNA polymerase
<i>POLG2</i>	PEO	17q23-24	Pol γ accessory subunit
<i>PEO1 (Twinkle)</i>	PEO/ataxia	10q24	Mitochondrial DNA helicase
<i>MGME1</i>	PEO, MtDNA depletion	20p11.23	RecB type exonuclease
<i>ANT1</i>	PEO	4q34-35	Adenine nucleotide translocator
<i>TP</i>	MNGIE	22q13.32	Thymidine phosphorylase
<i>DGUOK</i>	MtDNA depletion	2p13	Deoxyguanosine kinase
<i>TK2</i>	MtDNA depletion	16q22	Mitochondrial thymidine kinase
<i>MPV17</i>	MtDNA depletion	2p21-23	Mt inner membrane protein
<i>SUCLA2</i>	MtDNA depletion	13q12.2-13.3	ATP-dependent Succinate-CoA ligase
<i>SUCLG1</i>	MtDNA depletion	2p11.2	GTP-dependent Succinate CoA ligase
<i>RRM2B</i>	MtDNA depletion	8q23.1	p53- Ribonucleotide reductase, small subunit
<i>OPA1</i>	Dominant optic atrophy	3q28-29	Dynamin related GTPase
<i>FBXL4</i>	MtDNA depletion, Encephalopathy	6q16.1-16.3	Mitochondrial LLR F-Box protein

Table 2Major clinical syndromes associated with *POLG* mutations.

Syndrome	Age of Onset	mtDNA defect
Myocerebrohepatopathy spectrum (MCHS)	Neonatal/Infancy	Depletion
Alpers-Huttenlocher syndrome (AHS)	Infancy/Childhood	Depletion
Ataxia neuropathy spectrum (ANS)	Adolescent/young adult	Deletions
Myoclonus, epilepsy, myopathy, sensory ataxia (MEMSA)	Adolescent/young adult	Deletions
Progressive external ophthalmoplegia (PEO) with or without sensory ataxic neuropathy and dysarthria (SANDO)	Adolescent/young adult	Deletions