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Review

Mechanisms regulating mitochondrial DNA quality control

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Abstract

Maintenance of genome integrity is essential for cellular survival. There are mechanisms utilized by the cells to sense and respond to assaults on genomic DNA. These mechanisms are conserved across all domains of life and are collectively called the DNA damage response pathways. However, eukaryotic cells also have extrachromosomal DNA in mitochondria (mtDNA), which is indispensable for mitochondrial function, and hence cell survival. Indeed, impaired mitochondrial activity arising due to mutations in mtDNA has been found to be associated with many human pathologies. Despite its importance, our understanding of how cells ensure mtDNA genome integrity is limited. Since mitochondria do not encode for machinery required for the maintenance of their own genomes, they depend on the nucleus for replication, transcription, and repair processes. This adds a layer of complexity with the requirement for organelle crosstalk and coordination in response to mtDNA damage. This review summarizes recent findings that provide new insights into mechanisms involved in mtDNA quality control, acting at the level of mtDNA or organelle and also discusses a few new avenues of research towards a comprehensive understanding of the "mtDNA damage response".

Keywords: mtDNA damage, DNA damage response, mtDNA repair, mitochondria, mitophagy

INTRODUCTION

Preservation of genome integrity is essential for the faithful propagation of life. Sources of DNA damage include endogenous factors such as errors during DNA replication, reactive oxygen species (ROS), and metabolic intermediates (like methylglyoxal), as well as exogenous agents including UV radiation and chemicals that can directly modify and damage the DNA[1,2]. Responses to such perturbations require mechanisms that can sense damage and repair aberrant DNA modifications. In eukaryotes, this entails sensing and responding to both nuclear and mitochondrial DNA (mtDNA) damage.

All eukaryotic cells, with a few exceptions, contain mitochondria which are double membrane-bound organelles. While chiefly the sites for oxidative phosphorylation, mitochondria are also involved in other cellular processes, including amino acid biosynthesis, Fe-S cluster biogenesis, and apoptosis[3-5]. Mitochondria are highly dynamic and undergo changes in shape and size throughout the cell cycle, independent of cell division[6]. Apart from playing an important role in cellular adaptation to different metabolic environments[7], this dynamicity is important for quality control, segregation, and distribution of the organelle as well as its DNA[7,8].

Mitochondria possess their own genomes (mtDNA), which are essential for mitochondrial function. These genomes vary drastically in size as well as numbers across eukaryotes[9]. For example, yeast mitochondrial genome is ~86 Kb in size and is present in ~10-80 copies per cell[10,11], while human mtDNA is ~16.5 Kb, and present in a few hundred to thousand copies per cell[12,13]. Mitochondrial genomes code for proteins that are part of the electron transport chain as well as tRNA and rRNA genes required for mitochondrial protein translation[10,14,15]. The DNA copies are packaged in the form of nucleoids, which are bound by many proteins involved in replication, transcription, and repair[16,17]. The most abundant of these is an HMG (high mobility group)-box containing protein, TFAM (Transcription factor A, mitochondrial in metazoans) or ABF2 (in budding yeast), which functions in the regulation of mtDNA packaging, transcription, and replication[18-23].

Similar to nuclear DNA, mtDNA also experiences damage. For example, studies have demonstrated that mtDNA faces relatively more oxidative damage than nuclear DNA[24,25], likely due to the proximity of mtDNA to the OXPHOS machinery[26]. Another source of perturbation to mtDNA is the intrinsic errors from mtDNA replication, which can result in mutations or deletions[27]. mtDNA mutations are associated with several human pathologies like LHON (Leber hereditary optic neuropathy), MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes) syndrome, Leigh syndrome, *etc.*[28], and have been proposed to be an underlying cause for aging as well[29]. Thus, the maintenance of mtDNA integrity is critical for cell survival. How do cells sense and respond to mtDNA damage? Nuclear DNA damage triggers dedicated DNA damage responses that include cell cycle regulation as well as expression of genes directly involved in DNA repair[30]. Such a response in case of mtDNA damage remains to be uncovered. However, recent evidence suggests that cells do respond to mtDNA perturbations at two levels: DNA (via engaging in mtDNA repair or clearance) and organelle (via selective segregation or organelle clearance)[31-36]. The presence of multiple mechanisms that work on different levels adds complexity, as each pathway could have different consequences for the organelle and cell physiology. We point readers to excellent reviews covering topics of mtDNA damage repair, mitochondrial dynamics, and mitophagy in detail[6,8,37-43]. For this review, we limit our discussion to a few key studies that allow us to synthesize our broad understanding of mtDNA damage response and repair across scales (mtDNA, mitochondrial and cellular aspects). We discuss various pathways in light of their involvement in mtDNA quality control and highlight open areas to uncover a potential mtDNA damage response and downstream regulators of pathway choice [Figure 1].
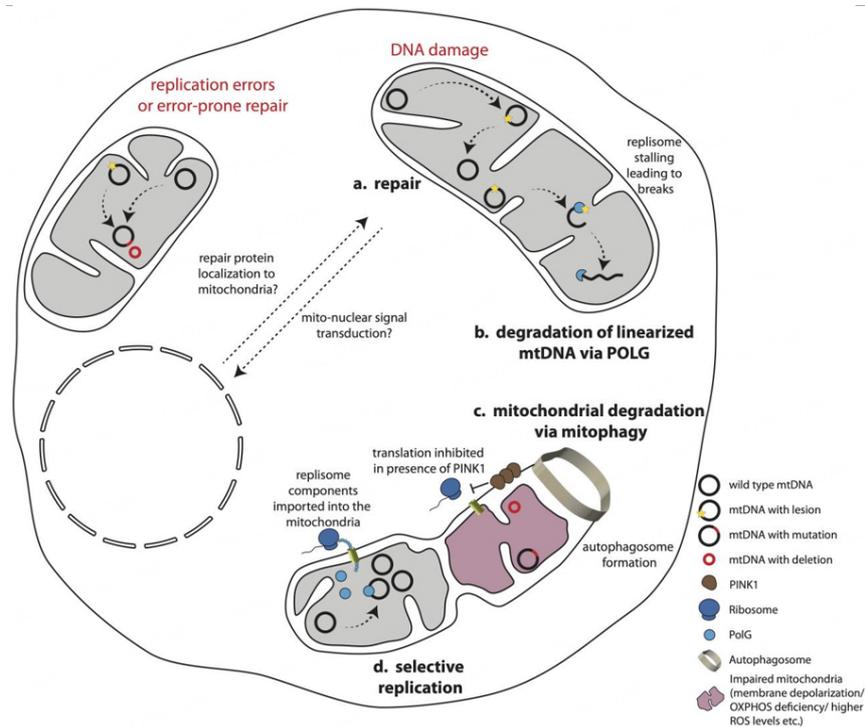
Figure 1. Sources of mtDNA perturbations include DNA damage and errors associated with replication machinery resulting in mutations or deletions. Different mechanisms involved in mtDNA quality control: a: mtDNA damage repair, b: mtDNA degradation via POLG, c: degradation of mitochondria containing mutated or damaged mtDNA via mitophagy, or d: selective replication of wild-type mtDNA. Each of these mechanisms (with literature references) is discussed in detail in the main text.

MTDNA QUALITY CONTROL: MTDNA REPAIR

There are several lines of evidence in support of proficient mtDNA damage repair across eukaryotic model systems[37-39]. Interestingly, these studies suggest that only certain types of damage might be repaired in mitochondria, with some classes of base modifications being tolerated efficiently by the replicative mtDNA polymerase, POLG/MIP1[44-47]. For example, the incorporation of rNMPs on mtDNA does not affect POLG stability or fidelity[44,47]. Indeed, there are also repair pathways that appear to be absent from mitochondria. For example, there is presently no evidence for mitochondrial nucleotide excision repair (NER)[48,49]. We direct readers to these excellent recent reviews for detailed repair pathway descriptions[37-39,50], and we highlight some major mechanisms here.

Base excision repair (BER) seems to be the most active repair pathway in mitochondria. The prevalence of BER can be appreciated by the fact that in somatic cells, the mutation landscape of mtDNA is devoid of G to T mutations, which are hallmarks of oxidative damage[51,52]. In support of BER activity on mtDNA, several DNA glycosylases have been found to localize to the mitochondria[53-59]. This includes OGG1 (8-oxoguanine DNA glycosylase) required for repair of 8-oxo-G lesions and UDG or UNG (Uracil DNA glycosylase) essential for removal of misincorporated uracils on DNA[53,58]. Indeed, inhibition of UNG1 activity leads to the accumulation of mutations on mtDNA in budding yeast[53]. Other than OGG1 and UNG1, two homologs of the human NTH1 (Endonuclease III-like protein 1), NTG1, and NTG2 with broad spectrum specificities, are also found in budding yeast. NTG1 dually localizes to both the nucleus and mitochondria[59-61] and preferentially translocates to the mitochondria under elevated mitochondrial oxidative stress[61]. Apart from NTH1, human cells also contain NEIL1 and NEIL2 glycosylases which are bi-functional and also have broad-spectrum specificities[55-57]. Some other glycosylases, such as MYH, could

have tissue-specific mitochondrial expression, as shown in rat brains[54]. The idea that BER is fully active in mitochondria is further supported by the presence of accessory proteins, such as endonucleases, required in this pathway, including APE1, FEN1, and DNA2[62-66]. APE1 is required for hydrolyzing the phosphodiester bond at the apurinic/apyrimidinic site to generate a gap that is then filled by a polymerase[67,68]. While the mtDNA replicative polymerase POLG is known to participate in gap-filling, there is now increasing evidence to suggest that X family polymerase, POLβ, is involved in this gap filling in mammalian mitochondria[69-72], with evidence for brain tissue-specific localization[69]. The choice of polymerase for gap-filling could depend on the type of lesion and glycosylase recruited. For example, POLβ also has ~17-20 fold higher 5′-dRP lyase activity as compared to POLG[71,72], and this activity is required for the processing of ends generated by mono-functional glycosylases such as UNG1 or MUTYH along with APE1. Alternately, in scenarios where the lesion is acted upon by bi-functional glycosylases such as OGG1, NEIL1, or NEIL2, the ends generated can be processed by either TDP1, APTX, or PNKP (polynucleotide kinase 3′-phosphatase)[57,73,74]. Following polymerization, ligation during mitochondrial BER requires DNA Ligase III[75].

There is some evidence to suggest that translesion synthesis may also be active in mitochondria. There are several translesion synthesis (TLS) polymerases such as POLθ, REV1, and POLζ that localize to mitochondria[76], and affect mutation rates[77-79]. Apart from TLS, it is possible that these polymerases also participate in BER as observed in *in vitro* assays[80,81], but *in vivo* evidence for the same remains to be uncovered. Indeed, TLS itself can be carried out by POLG, which has been shown *in vitro* to synthesize across lesions like pyrimidine dimers and acrolein-derived exocyclic DNA adducts[82,83].

Apart from BER and TLS, there is sporadic evidence for the presence of other damage repair and tolerance systems in mitochondria. This includes mismatch repair[84] and double-strand break (DSB) repair[33,85,86]. While DSB repair is known to occur in plant mitochondria[87-90], it was widely believed to be non-existent in the mitochondria of animal cells. However, studies have shown that mitochondrial extracts obtained from mammalian cells are capable of DSB repair via HR (homologous recombination) or MMEJ (Micro-homology mediated end joining)[85,86,91,92]. These *in vitro* assays show end-joining activity in the mitochondrial extracts, leading to the formation of repair products with deletions[86,91]. Indeed, in mouse muscle cells, expression of a mitochondrial-targeted restriction enzyme, PstI, resulted in the formation of recombination products with large deletions, albeit at low frequencies[93]. Similarly, mitochondrial targeting of restriction enzyme, ScaI, in heteroplasmic mouse cells containing mtDNA haplotypes with different numbers of Sca1 restriction sites, resulted in the formation of both inter- and intra-molecular recombination products, with low levels of inter-molecular recombination[94]. In support of this, low frequencies of inter-molecular recombination products were also observed after the fusion of two human cytoplasmic hybrids with different mtDNA mutations[95]. In a similar study, the fusion of cybrids with mtDNA bearing different deletions allowed for functional complementation, but the genetic exchange between the two mtDNA nucleoids occurred at low frequencies[96]. More recently, using heteroplasmic mtDNA variants in *Drosophila*, recombination was shown to occur *in vivo* after induction of mtDSBs using mitochondrial-targeted restriction enzymes[97], and a screen for candidates involved in mtDNA repair revealed REC/MCM8 as a candidate involved in recombination-mediated repair[33]. Thus, these pieces of evidence show that DSB repair can occur in the mitochondria of animal cells. Future studies in this direction can further elucidate the importance of this pathway in mtDNA integrity maintenance under physiological conditions.

A particularly confounding feature of mtDNA damage is the fact that there are multiple copies of mtDNA in the cell. Thus, in the case of mtDNA damage, cells can choose between either repairing the damage or

degrading the damaged DNA copy, followed by replication of wild-type mtDNA to restore copy number. Indeed, in case of mutations/deletions, clearance of damaged DNA copies and/or selective replication of intact mtDNA might be the only possible way of quality control. Whether damage on mtDNA is repaired or degraded could thus depend on the type of DNA damage as well as the amount of damage accumulated.

MTDNA QUALITY CONTROL: MTDNA CLEARANCE AND SELECTIVE REPLICATION

As an alternative to repair, multiple pieces of evidence show that in cases where damage cannot be repaired or is persistent, the mtDNA is degraded[31,98,99]. For example, UV-induced dimerization of pyrimidines, or ADP ribosylation on mtDNA leads to degradation of DNA[31,100,101]. In support, there are no reports of NER in mitochondria, which would be required for the repair of such lesions[48,49]. Even in the case of DNA breaks, mtDNA copies with DSBs are degraded and copy number is replenished by replicating wild-type mtDNA[98]. Studies utilizing mitochondrially-targeted restriction enzymes, TALENS, and ZFNs, also show that DSBs on mtDNA result in degradation, and these have been utilized to reduce the levels of heteroplasmy[102-106] [Table 1].

How is mtDNA degradation carried out? Although multiple nucleases have been detected in the mitochondria, these do not appear to play a role in mtDNA degradation[98]. Instead, recent studies have shown that the mitochondrial replicative polymerase, POLG/MIP1, is capable of degrading mtDNA under damage or even in starvation conditions[31,98,123,124]. A role for MGME1 in the degradation of mtDNA has also been suggested, where the loss of MGME1 prevents the degradation of linearized mtDNA after induction of DSBs[98]. However, an alternate set of observations suggest that loss of MGME1 results in the *de novo* formation of linearized DNA fragments due to incomplete mtDNA replication and does not affect the degradation of mtDNA[125,126]. These studies suggest that POLG might play the primary role in the degradation of linearized mtDNA. However, how MIP1/POLG switches from a replicative to an exonuclease function is not fully understood. There is evidence suggesting that this switching is regulated by the levels of dNTPs in the cell[123,127]. Whether this results in the selective degradation of few mtDNA copies or "global" degradation of all mtDNA is not known. For example, it is possible that polymerase stalling at a DNA lesion triggers its exonuclease function[128,129], resulting in the degradation of only the damaged mtDNA copy. Such a mechanism would nicely couple mtDNA replication with genome integrity maintenance, and prevent unregulated DNA loss in case of damage. Indeed, selective degradation of damaged mtDNA would also contribute to selective mtDNA clearance as well as purifying selection via replication of non-damaged mtDNA.

Certainly, a combination of selective degradation and replication could contribute to mitochondrial genome integrity maintenance. This becomes particularly relevant in conditions of heteroplasmy[130]. In line with this, there is evidence to support that wild-type mtDNA is selectively replicated over mutant mtDNA in *Drosophila* cells[131-133]. For such selective replication to occur, cells would need to distinguish wild-type mtDNA from mutant or damaged mtDNA. As stated above, this could come from the replicative polymerase stalling at lesions, followed by selective degradation of that specific mtDNA copy. In addition to mtDNA-specific mechanisms, there is strong evidence for the regulation of selective degradation/ replication occurring at the level of the mitochondria itself.

Recently, local protein translation was shown to be dependent on mitochondrial membrane potential in *Drosophila* germline cells[133]. PINK1 accumulation on depolarized mitochondria prevents protein translation on the outer mitochondrial membrane, thereby reducing the abundance of proteins (including replisome components) in mitochondria[133]. Similarly, mitochondria with lower membrane potentials cannot undergo fusion[134-136], and mitochondrial fusion is known to affect mitochondrial DNA replication in

Table 1. Different tools for specifically modifying mitochondria DNA

Tool	Mode of action	Type of modification	References
Mito-ZFN (Zinc finger nucleases)	Zinc finger nucleases are targeted to mitochondria using a mitochondrial targeting sequences (MTS). The conventional design consists of the catalytic domain of type IIS restriction enzyme *Fok1* attached to a specially designed Zinc Finger Peptide (ZFP) which gives sequence specificity.	Double-strand break	[105]
Mito-TALENs (TALE based nucleases)	Work on the same principle as ZFNs; however, sequence specificity is brought about by a TALE (transcription activator- like effectors) instead of ZFPs.	Double-strand break	[104,106,107]
Mito-Tev1-TALE or cTALEN (c:-compact)	Utilizes a T4 phage homing endonuclease I-Tev1 instead of *Fok1*. I-Tev1, unlike *Fok1*, does not require dimerization for cutting DNA.	Double-strand break	[108,109]
Mitochondrial targeted restriction endonucleases Mito-*Xmal* Mito-*Smal* Mito-*Pstl* Mito-*Scal* Mito-*ApaLl* Mito-ARCUS	Restriction endonucleases are targeted to mitochondria using a MTS. They cut DNA at specific recognition sites on mtDNA.	Double-strand break	[102,103,110-113]
Mito-DarT	Utilizes a bacterial toxin DarT from a bacterial toxin-antitoxin system, which is targeted to mitochondria using a MTS.	ADP ribosylation on *ss*DNA on a thymidine residue in a "TNTC" motif	[31]
Triphenyl phosphonium (TPP) conjugates TPP-Doxorubicin TPP-Chlorambucil TPP-Cisplatin TPP-Paraquat	TPP localizes to mitochondria in a membrane potential sensitive manner.	Doxorubicin- Double strand breaks Chlorambucil- alkylation Cisplatin- Crosslinks Paraquat- Increase in ROS levels	[114-117]
Mitochondria penetrating peptides (MPPs) conjugates MPP-Doxorubicin MPP-Cisplatin MPP-Chlorambucil	MPPs are cationic, but lipophilic peptides whose penetration into mitochondria has been observed and this localization can be fine-tuned based on charge and lipophilicity.	Doxorubicin- Double strand breaks Cisplatin- Crosslinks Chlorambucil- alkylation	[118-120]
Mitochondrial targeted Topoisomerase 1 mutant: *TOP1-103* (R420K)	A substitution of arginine to lysine at position 420 of TOP1 results in a toxic mutant causing persistent single-strand breaks.	Single strand break	[121]
Mito-FAP (mitochondrially targeted fluorogen-activating peptide)	Mito-FAP-MG-2I (Mito-FAP bound to iodine-substituted malachite green analog) complex generates singlet oxygen in the presence of NIR (near-infrared light).	Oxidative damage (Leads to an overall increase in ROS levels in the cell. Could damage nuclear DNA as well)	[122]

both mammalian and budding yeast cells[137,138]. Given that preventing mitochondrial membrane fusion by MFN1 and MFN2 or OPA1 knockout resulted in an imbalance of replisome components (like POLG, TWINKLE, and SSBP1) in mitochondria[138], a mechanism for such reduced mtDNA replication can be hypothesized. However, for this mitochondrial activity-driven effect to dictate specificity in replication, the influence of mutations or damage on protein quality would need to be localized in the close vicinity of the damaged nucleoid(s). In support of this idea, in budding yeast, mtDNA-encoded ATP6 was shown to have limited diffusion, depending on cristae morphology[139]. This would result in the compartmentalization of mutant mt-nucleoids as well as its effects on mitochondrial activity. Such compartmentalization could further contribute to the selective shutdown of replication in these regions, driving selective DNA replication of wild-type mtDNA copies instead.

It is important to note that there are examples that are counter to the mechanism of selective replication of wild-type mtDNA described above. In contrast to *Drosophila*, where PINK1 localization to depolarized mitochondria suppresses mutant mtDNA replication[133], studies in *C. elegans* have shown an expansion of

mtDNA carrying deletions[140,141]. This expansion is dependent on the activation of the UPR^mt (mitochondrial unfolded protein response), resulting from OXPHOS dysfunction[142]. Activation of the UPR^mt leads to accumulation of ATFS-1 in mitochondria with impaired OXPHOS machinery, finally resulting in increased binding of POLG on mtDNA[143]. Furthermore, active replication is probably not the only factor regulating selectivity; even within a cell that only has wild-type mtDNA, not all DNA copies are replicated[144]. In such instances as well, mtDNA damage will affect mitochondrial function and cells must have additional mechanisms to cope with the same.

MITOCHONDRIAL QUALITY CONTROL UNDER CONDITIONS OF MTDNA DAMAGE

An important layer of mitochondrial genome integrity maintenance appears at the organelle level. As discussed in the previous section, the effect of mtDNA perturbation can adversely impact local mitochondrial activity as well[139]. This in turn can activate quality control mechanisms that function at the level of the organelle. Multiple mitochondrial quality control pathways have been shown to function in response to mitochondrial stress, including membrane depolarization, proteotoxic stress, or ROS accumulation, among others[145-147]. A key step in this process is the re-organization of the mitochondrial network to physically separate impaired and fit mitochondria[148]. Both mitochondrial fission and fusion play essential roles in re-organizing the mitochondrial network[40,145]. Indeed, mitochondrial fragmentation has been observed in response to both mtDNA damage and the presence of mtDNA with mutations[31,32,34]. This is then accompanied by either a. mitochondrial clearance and/ or b. selective mitochondrial segregation [Figure 2].

1. Active clearance of mitochondria mainly occurs via a process called mitophagy (mitochondria-specific autophagy)[41]. Other recently discovered mechanisms that help in mitochondrial protein quality control are via the formation of MDVs (Mitochondria Derived Vesicles) and SPOTs (Structures Positive for Outer Membrane), which are then actively degraded in the cell[149-151]. Clearance of any impaired mitochondria via such mitochondrial quality control pathways could passively clear out mtDNA with mutations or damage as well. In support of this mechanism, in *Drosophila* germline cells, clearance of deleterious mutations is dependent on the fragmentation of the mitochondrial network[34]. Fragmented mitochondria are ultimately degraded via mitophagy[34]. Similarly, even in mammalian cell lines, mtDNA damage-associated fragmentation is followed by activation of autophagy[32]. In *Drosophila*, such mitophagy appears to be independent of the mutation load and is programmed to occur at a specific stage of development[35]. Even in somatic cells in *Drosophila* and *C. elegans*, activation of the PINK1/Parkin pathway under lower levels of Mitofusin results in purifying selection of mtDNA[152-154]. These data suggest a role for mitophagy in conjunction with mitochondrial fragmentation in mtDNA purification.

2. Additionally, separation of impaired and fit mitochondria could also allow for selective segregation of organelles during cell division, independent of mitophagy. For example, in budding yeast, it has been found that "fitter" mitochondria are segregated into the daughter cells, while "unfit" mitochondria are retained in the mother cell[155]. Fitness, in this scenario, is defined in terms of ROS levels, presence of protein aggregates, and redox states of the mitochondria[155]. During division, daughter cells receive mitochondria with lower ROS levels and higher redox states, thereby also generating mother-daughter age asymmetry[155]. Such an asymmetry in mitochondrial segregation could contribute to selective segregation of mtDNA, without the need for active degradation. In support, recent work suggests that under mtDNA damage, mitochondrial fragmentation is not followed by mitophagy, and instead, this fragmentation could facilitate asymmetric segregation of the organelle[31]. Indeed, even in adult stem cells, segregation of mitochondria during division is asymmetric, where one cell receives "old" mitochondria whereas the other cell receives "young" mitochondria[156,157]. Whether this also translates to mtDNA quality control in these mitochondria remains to be assessed.
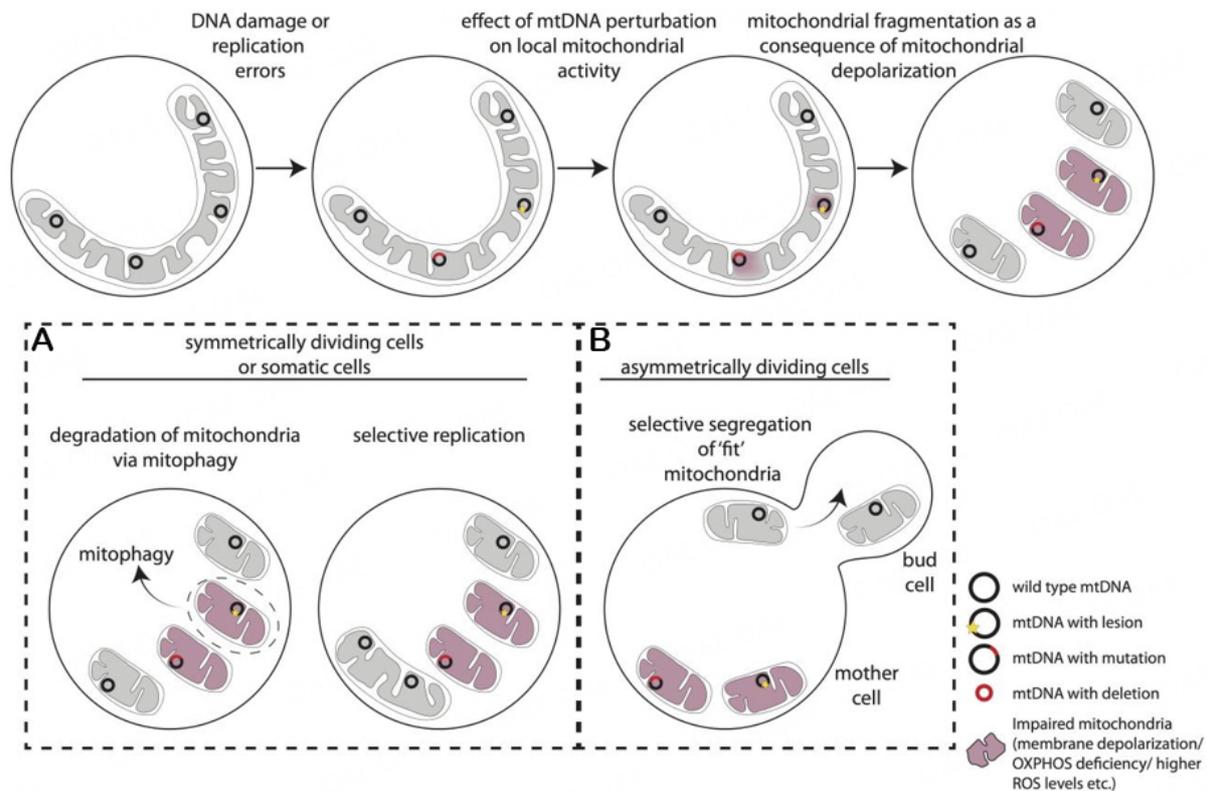
Figure 2. Fragmentation of mitochondrial network results from perturbed mitochondrial activity to compartmentalize mitochondria with impaired function. These fragmented mitochondria have different fates: (A) active clearance via pathways like mitophagy and (B) selective segregation with retention of impaired mitochondria in one cell.

FUTURE PERSPECTIVES

It is becoming increasingly evident that mtDNA quality control is important and that diverse mechanisms act at the level of mtDNA as well as mitochondria to regulate the same. However, key questions about the relative importance of these mechanisms, the choice of pathway employed, and the cellular impact of the pathway(s) used remain to be answered. A previous lacuna in the field was the challenge associated with specifically perturbing mtDNA integrity, avoiding confounding effects on nuclear DNA. The toolkit to generate specific types of DNA modifications on mtDNA is now expanding [Table 1] and will enable researchers to disentangle the roles played by DNA repair, degradation, and organellar regulation in the maintenance of mtDNA integrity. More importantly, these tools can address whether cells encode an mtDNA-specific damage response, such as those mounted in response to nuclear DNA damage[30]. In this direction, we highlight the following open questions:

(1) How is mtDNA damage sensed?

(2) How is the damage signal relayed from mitochondria to the nucleus?

(3) What is the machinery involved in carrying out this sensing and relaying?

In the case of nuclear DNA damage, cells sense damage via ATM/ATR proteins which are recruited at the site of damage following replication or transcription blocks[158,159]. It is unclear whether mtDNA damage also activates such a response. Indeed, some studies show localization of ATR in mitochondria where it plays an anti-apoptotic role[160]. There is also evidence that CHK2 activation can affect mtDNA synthesis, independent of mtDNA damage[161]. However, the involvement of such responses in mtDNA damage is not known. Koczor *et al.*, did observe CHK2 activation in response to increased ROS production in HeLa cells[162]. This response was deactivated when mtDNA-associated BER proteins were overexpressed, suggesting a link between mtDNA integrity and DNA damage checkpoint activation. The mechanism of activation of this response via mitochondrially-relayed signals (if any) and what is the functional significance of the same remains to be determined. Identification of the mechanism of this activation would reveal new insights into mitochondria-nuclear signaling and crosstalk.

Recent studies suggest that mtDNA damage can also trigger other responses in the cell. For example, mtDSBs have been shown to activate the integrated stress response via the OMA1-DELE1-HRI pathway[163]. In addition, mtDNA stress can also result in the release of mtDNA and/ or mtRNA into the cytosol[36,164-168]. This has been shown to activate the type-1 interferon response via the cGas-STING pathway (in case of mtDNA) and RIG1/MDA-5-MAVS pathway (in case of mtds-RNA)[36,164-168]. In some of these cases, there appears to be a direct connection between the regulation of mitochondrial cristae morphology and signal transduction from the mitochondria to the cytosol, likely mediated by ATAD3[163,167]. ATAD3 is required for the attachment of mtDNA to the inner mitochondrial membrane and is also implicated in the regulation of cristae morphology[169-171]. The central role of ATAD3 in mitochondria-nuclear signaling under mtDNA stress would suggest that inner membrane architecture could be a potential marker for mtDNA dysfunction[172,173]. It would be insightful to assess whether other mtDNA structuring and organizing proteins[174,175] also contribute to such signaling mechanisms.

Taken together, even at the level of signaling, there appear to be contributions at the level of mtDNA and the organelle. As discussed above, it is possible that the responses to mtDNA perturbations vary dependent on the amount and type of mtDNA damage faced by the cell, as well as growth conditions and cellular contexts[31,32,163,176]. Careful delineation of these mechanisms can reveal the general principles of an mtDNA damage response as well as specific context-dependent features that could contribute to pathway choice and associated heterogeneity in purifying selections[177]. Ultimately, these insights can enable us to target mtDNA quality control mechanisms in pathological conditions.

DECLARATIONS

Authors' contributions
Conceptualized this review and contributed to the writing of the manuscript: Dua N, Badrinarayanan A

Availability of data and materials
Not applicable.

Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
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