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REVIEW

Turnover and Quality Control of Mitochondrial DNA

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Abstract—The quantitative content of mitochondrial DNA (mtDNA) – a multicopy circular genome – is an important parameter relevant for function of mitochondrial oxidative phosphorylation (OxPhos) in cells, since mtDNA encodes 13 essential OxPhos proteins, 22 tRNAs, and 2 rRNAs. In contrast to the nuclear genome, where almost all lesions have to be repaired, the multicopy nature of mtDNA allows the degradation of severely damaged genomes. Therefore, cellular mtDNA maintenance and its copy number not only depend on replication speed and repair reactions. The speed of intramitochondrial mtDNA degradation performed by a POLGexo/MGME1/TWNK degradation complex and the breakdown rate of entire mitochondria (mitophagy) are also relevant for maintaining the required steady state levels of mtDNA. The present review discusses available information about the processes relevant for turnover of mitochondrial DNA, which dysbalance leads to mtDNA maintenance disorders. This group of mitochondrial diseases is defined by pathological decrease of cellular mtDNA copy number and can be separated in diseases related to decreased mtDNA synthesis rates (due to direct replication defects or mitochondrial nucleotide pool dysbalance) or diseases related to increased breakdown of entire mitochondria (due to elevated mitophagy rates).

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Keywords: mtDNA maintenance, mtDNA replication, mtDNA degradation, determinants of cellular mtDNA content, mtDNA maintenance disorders

mtDNA REPLICATION MECHANISM

Human mitochondria contain a 16.5 kb circular mitochondrial genome, which comprises the genetic information for 13 proteins of the mitochondrial inner membrane (subunits of OxPhos complexes), 22 tRNAs and 2 rRNAs. *In vitro* experimental work showed that the basic mitochondrial replisome required for replication of this genome consists of the mitochondrial polymerase POLG, the mitochondrial single-stranded DNA-binding protein (mtSSB), and the replicative helicase TWINKLE [1]. Although three different models of mitochondrial DNA (mtDNA) replication have been proposed (for a comprehensive overview of all three models cf. [1]), recent scientific literature favors the classical asynchronous strand-displacement replication model of mtDNA [2], initially proposed by the group of Vinograd [3] and later on verified by experimental work of Clayton and coworkers [4, 5]. As shown in Scheme 1, according to this model replication is initiated at the OriH and replication of the H-strand proceeds along 70% of the mtDNA until the OriL is exposed and replication of the L-strand is initiated and proceeds in the opposite direction [5]. Newer relevant data in support of this replication model include (i) the conservation of OriL, (ii) the occupancy of mitochondrial single-stranded DNA binding protein, (iii) the mtDNA point mutation profile, and (iv) the pattern of mtDNA ends detected by ultra-deep long-read sequencing.

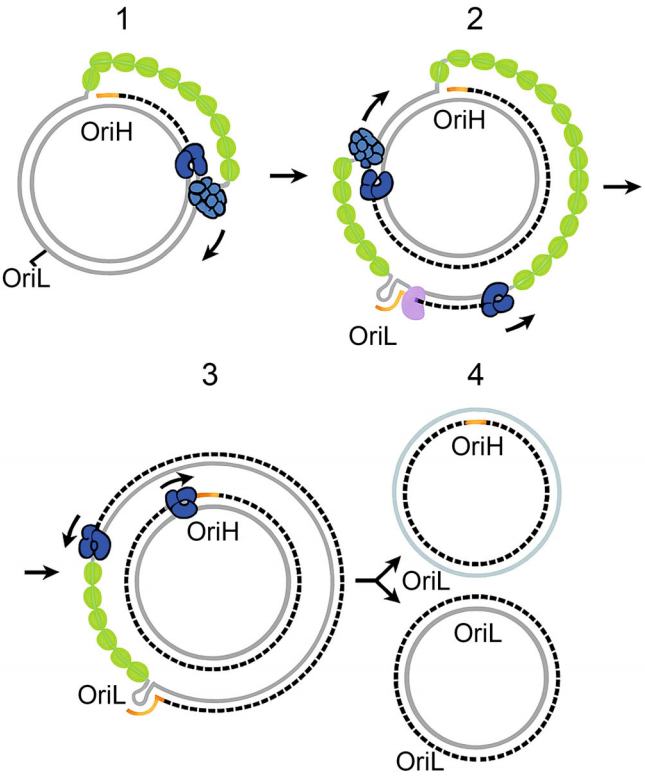
(i) CONSERVATION OF OriL

Using in vivo mutagenesis techniques Wanrooij et al. [6] showed that OriL is indispensable for mtDNA replication. When OriL is altered or deleted, mtDNA

replication is severely impaired, underscoring its essential function. The conservation and essentiality of OriL strongly support the classical strand-displacement model, as it shows that replication cannot proceed effectively without it.

(ii) *In vivo* OCCUPANCY OF SINGLE-STRANDED DNA BINDING PROTEIN

Fusté et al. [7] investigated how mtSSBs interact with mtDNA during replication. mtSSBs selectively bind to single-stranded regions of mtDNA *in vivo*, particularly in the regions exposed during strand-displacement replication. This binding stabilizes the displaced single strand of DNA and protects it from damage while replication proceeds on the opposite strand. In the strand-displacement model, as the replication fork progresses, one strand of DNA becomes temporarily single-stranded. The binding of mtSSBs to these regions is a key feature, as it prevents degradation or secondary structure formation in the displaced strand. This study provides direct evidence for mtSSBs' role in maintaining strand stability in mitochondria, validating that strand-displacement occurs and requires specific proteins to protect and to stabilize the single-stranded regions.



Scheme 1. Strand-displacement model of mtDNA replication (modified according to a scheme presented in [1]). 1) Replication of the nascent H-strand (dashed circle) is initiated at OriH and proceeds unidirectionally by POLG/POLG2 (dark blue) and unwinding by TWNK (light blue). In the process, the parental H-strand is displaced and covered by mtSSB (green). 2) When the replication machinery reaches OriL, the H-strand of the origin folds into a stem-loop structure. POLRMT (purple) initiates from the poly-dT stretch in the loop region a short RNA primer (orange) that is used to initiate L-strand DNA synthesis. 3) The nascent L-strand is synthesized continuously until full-circle and two new full-length circular daughter molecules are formed. 4) The synthesis of one daughter molecule containing the nascent H-strand is initiated and terminated at OriH, whereas synthesis of the other daughter molecule is initiated and terminated at OriL. For removal of 5′ overhangs from the primers and formation of ligatable ends MGME1 is required [89]. The end ligation of the nascent strands is performed by LIG3 and at OriH potentially also by TOP3A [9].

(iii) mtDNA POINT MUTATION PROFILE

Recent research by Iliushchenko et al. [8] focused on the mutation patterns within mtDNA across chordates. The authors observed that some mutations are linked to DNA damage, while others appear to arise from replication-specific processes. For instance, regions frequently exposed as single strands during strand-displacement replication may accumulate mutations due to increased vulnerability to damage or errors in replication. The presence of these replication-induced mutation profiles aligns with the strand-displacement model, as it implies that single-stranded regions are exposed regularly enough to leave a mutational "signature." This provides indirect support for the strand-displacement mechanism by linking observed mutation patterns to replication dynamics.

(iv) PATTERN OF mtDNA ENDS DETECTED BY ULTRA-DEEP LONG-READ SEQUENCING

Sensitive end detection by ultra-deep long-read sequencing of native and nuclease S1-treated isolated mtDNA confirmed in wild-type HEK293 cells the absence of Okazaki fragment related ends and provided evidence for the presence of replication intermediates having ends in the OriH, TAS, and OriL regions [9]. This result can be explained in the framework of the classical replication model of mtDNA [3] only.

Together, these newer findings collectively affirm the asynchronous strand-displacement model by showing how specific origins, stabilizing proteins, replication-linked mutation patterns and detected replication intermediates align with this particular replication mechanism of mitochondrial DNA.

REPLICATION SPEED AND TURNOVER MEASUREMENT OF mtDNA

Results from pulse-chase experiments indicate that mtDNA in mouse L cells takes about 120 min to

Table 1. Half-life of mtDNA species in cell culture

Mitochondrial constituent	Half-live	Enzymes involved	Methods	References
mtDNA 7S DNA	7-9 h	?	labelled nucleotides in mouse L-cells	[12]
	1 day	MGME1	ddC-induced mtDNA depletion in fibroblasts	[49]
	4 days	?	ddC-induced mtDNA depletion in MGME$^{-/-}$ fibroblasts	[49]
Linear mtDNA fragments	2-4 h	MGME1/POLGexo	fragment analysis in HEK cells	[37]
Total mtDNA	8-12 days*	?	labelled nucleotides in epithelial cells	[13]

Note. * Can be influenced by re-use of nucleotides.

complete one replication cycle [10]. This is relatively fast, representing only about 5% of the entire cell cycle. Unlike nuclear DNA that replicates synchronous with the cell cycle, mtDNA replication is not tightly coupled with nuclear DNA replication. This means mtDNA can replicate at different times, independent of the cell's nuclear DNA replication schedule, although imaging studies of synchronized HeLa cells detected that initiation of replication is coordinated with the cell cycle, preceding nuclear DNA synthesis [11]. The asynchronous replication allows mitochondria to produce more mtDNA whenever needed, which might be crucial for energy-demanding conditions in the cell. Interestingly, the 7S heavy-strand of mtDNA initiates more frequently, as indicated by a short half-life of 7-9 h [12]. It is part of a unique region in mtDNA called the D-loop, which is critical for starting mtDNA replication.

Labelling studies with ^{3}H-labelled nucleosides show that the half-life of mtDNA in relatively short-lived cells, such as epithelial cells or hepatocytes, is 8-12 days [13], whereas, in certain post-mitotic cells, such as neurons, the half-life of the mtDNA is 20-30 days. Postmitotic heart cells are an exception with a mtDNA half-live of 6-7 days [14]. Interestingly, under the investigated labeling conditions of heart cells no measurable turnover of nuclear DNA was noticed.

In the classical literature it has been reported that entire mitochondria show tissue-specific rates of turnover, if assessed by turnover of mitochondrial proteins, lipids or mtDNA. For example, in the mouse heart, mitochondria turn over with a half-life of 14 days [15], but in the liver, the half-life is 2-4 days [15, 16] and in brain 26 days [15]. This can be explained by selective mitochondrial autophagy, or mitophagy, which eliminates damaged and dysfunctional mitochondria [16-18] and is closely linked to mitochondrial biogenesis, which permits replacement of mitochondria (or synthesis of components and their insertion into the remaining functional mitochondria). Longer half-life of mtDNA in comparison to proteins might result from a re-use of the labelled nucleotides (cf. Table 1). The older literature data (cf. [15]) are in general agreement with the original hypothesis of Fletcher and Sanadi, who initially proposed mitophagy as main contributor to turnover of mitochondria. However, mitophagy of entire mitochondria obviously cannot explain the relatively large (approximately 10-fold) differences of turnover rates of individual proteins of the mouse proteome as determined by more advanced MS techniques using metabolic heavy water ($^{2}H_2O$) labeling [19]. As examples in mouse heart ATP5i and CS had a half-life of 54.1 days and 41.3 days, respectively, while OAT and DNAJC30 of 3.3 days and 4.4 days, respectively. In mouse liver the half-life of ATP5i and CS was 8.5 days and 7.5 days, respectively and of OAT 1.9 days [19]. With MS techniques remarkable differences in turnover were even detected for individual subunits of respiratory chain complex I: proteins of the N-module showed elevated turnover in comparison to the Q-module or the mitochondrially encoded subunits, which was strongly affected by mutations in the chaperone protein DNAJC30 [20].

DETERMINANTS OF mtDNA CONTENT IN CELLS

The mtDNA content in yeast cells as determined by a study of Seel et al. [21] depends on cell size, which directly influences the mtDNA amount which can change from 20 to 160 copies per cell. As cells grow, mitochondrial biogenesis is regulated to ensure mtDNA homeostasis, maintaining a consistent ratio relative to cell volume. This regulation ensures that energy production capacity meets cellular demands during growth. With cell size correlates the expression of the mitochondrial DNA polymerase Mip1 (the yeast POLG paralogue) and the yeast TFAM

paralogue Abf2 [21]. In yeast cells, other factors involved in mtDNA replication, like the helicase or the ssDNA-binding protein Rim1 had smaller effects.

In mammals, which contain a much more compact mitochondrial genome, the copy number of mtDNA (the mtDNA/nDNA ratio) is highly variable and is strongly cell type dependent: human sperm cells contain as little as 5-10 copies of mtDNA per nuclear genome [22] and in a recent study using digital droplet PCR even only 0.58 copies per nucleus were detected [23], which was attributed to a lack of functional TFAM. On the other hand, human oocytes may contain as many as 500,000 copies of mtDNA [24].

Overexpression of either Twinkle or TFAM in mice leads in mammals to a higher mtDNA amount and enhances recovery from ischemic heart damage [25-28] or delays of male infertility [29]. On the other hand, overexpression of DNA polymerase gamma (POLG), the mtDNA replicase, in contrast to yeasts does not affect mtDNA copy number in mammals, emphasizing the controlling role of the helicase Twinkle and TFAM for mammalian mtDNA replication and thus the cellular mtDNA content.

In addition to the replication process the copy number of mtDNA (the mtDNA/nDNA ratio) is obviously directly affected by biosynthesis of mitochondria. Here, nuclear transcription factors and coactivators play a critical role in mitochondrial biogenesis, particularly those in the PGC-1 (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1) family, as highlighted by Scarpulla [30, 31] and others. These nuclear factors integrate signals that promote mtDNA replication and transcription, adapting mitochondrial function to meet energy requirements. PGC-1α integrates the activity of many transcription factors, including NRF1, NRF2, and ERRα [30-32]. The expression of PGC-1α is significantly induced upon oxidative stress, in turn enhancing the expression of some antioxidant proteins, which can prevent excessive mitochondrial ROS production following mitobiogenesis [33]. Environmental stimuli, like cold exposure or training conditions can drive mitochondrial biogenesis. Wu et al. [34] describe how PGC-1 coactivators trigger thermogenic responses in brown adipose tissue, increasing mtDNA content and overall mitochondrial capacity as an adaptation to increased energy needs. Here, PGC-1α increases the transcriptional activity of NRF1, stimulates the production of NRF1 and NRF2, and increases the expression of TFAM and numerous mitochondrial respiratory chain genes, leading to biogenesis of mitochondria [34].

Thus, the cellular mtDNA content is tightly controlled by both intrinsic factors, like cell size, mtDNA replication speed and nuclear regulatory proteins, and extrinsic factors, such as environmental and metabolic stresses.

INTRAMITOCHONDRIAL mtDNA DEGRADATION

Due to the redundant nature and multiple copies of mtDNA, it is likely that intramitochondrial degradation of damaged mtDNA is the most efficient pathway to cope with double strand breaks (DSBs) or an overwhelming number of mutagenic lesions [35]. This is most probably the reason that mtDNA is rapidly degraded after extensive oxidative stress [36]. Interestingly, mtDNA replication proteins were found to be not only required for synthesis but also involved in the degradation of linear mtDNA fragments, specifically the 5′-3′ exonuclease MGME1 and the 3′-5′ exonuclease of POLG [37]. Since only blunt end double-stranded degradation intermediates have been identified after induction of mitochondrially targeted restriction enzymes [37] leading to defined DSBs, it has been proposed that both exonucleases – preferentially digesting single-stranded DNA molecules in separate directions – collectively work together on both strands. Interestingly, in all experiments which lead to the induction of DSBs in mtDNA blunt-end double stranded degradation intermediates with ends proximal to GC-rich regions have been identified [37, 38].

Degradation of mtDNA after DSBs, as opposed to end joining repair pathways present in the nucleus, avoids the formation of deletions [37, 39]. Intact mtDNA copies are then replicated to repopulate wild-type mtDNA species [40]. This has led to the idea of mtDNA being a “disposable” genome due to the multiple copies and the ability to repopulate independently of cell cycle [41], because degradation combined with the replication of intact copies is more efficient to maintain intact mtDNA rather than to invest in an error-prone repair [42]. Accordingly, all of the constituents of base-excision repair pathway have been identified in mitochondria [40], while double-strand break repair activities are apparently restricted to microhomology-mediated end joining only [43], which are implicated in the formation of pathogenic mtDNA deletions.

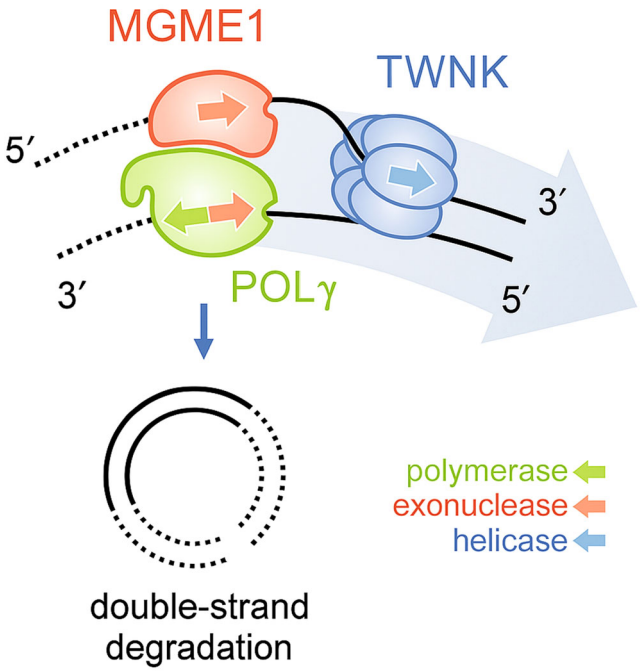
As stated above, the intramitochondrial degradation of linear dsDNA has been shown to depend on the action of two ssDNA specific exonucleases – the 3′-5′ exonuclease of POLG [37, 39] and MGME1 [37] – an exonuclease showing apparently a bidirectional ssDNA splitting behavior [44, 45]. Residual dsDNA splitting activity of MGME1 can be ruled on the basis of nuclease assays [44, 45] and its molecular mechanism – a ring-shaped architecture with a selective hole that allows only ssDNA to access the catalytic center [46]. For MGME1 a higher 5′-3′ activity with ssDNA substrates has been initially observed by classical nuclease assays [44, 47] and an approximately 3-fold 5′-3′ over 3′-5′ activity has been convincingly

confirmed by a fluorescence-based single molecule method [48]. To explain efficient degradation of long linear dsmtDNA fragments leading to the transient generation of shorter linear blunt-end dsDNA intermediates the action of two ssDNA specific enzymes with distinct direction preferences would be required. The concerted action of both exonucleases – MGME1 and POLGexo – as proposed in [37], fulfills these requirements. That also explains experimental data for additional requirement of the mitochondrial helicase TWINKLE [37], needed for unwinding of long linear dsDNA fragments.

Notably, a physical protein–protein interaction between a small fraction of POLG and MGME1 has been reported by high-resolution label-free mass spectroscopic and pull-down approaches in MGME1 overexpressing HEK293T cells [49]. These results [49] and the surprising observation that the lentiviral rescue of MGME1 in MGME1-deficient fibroblasts (which leads to a severe MGME1 overexpression) does not normalize mtDNA copy numbers, but causes severe mtDNA depletion [44] are compatible with the speculation that particularly the recruitment of MGME1 to POLG forms a degradation complex, suitable for efficient degradation of double-stranded mtDNA. The hypothetical detail of molecular events describing the degradation of *Eag*I- or *Pst*I-linearized mtDNA (as described in detail in [37]) or linear mtDNA fragments occurring after oxidative stress [38] can be outlined as follows:

1. DSB-lesion detection by POLG
2. Filling-in of short 5′ overhangs (of note: these overhangs are not degraded by MGME1!) or degradation of short 3′ overhangs by POLG to generate blunt ends
3. Recruitment of MGME1 and TWNK to the blunt ends, respectively
4. Formation of a degradation complex between the three proteins
5. Degradation of linear mtDNA with transient generation of blunt-end dsDNA intermediates due to the slowing down of degradation activity at GC-rich stretches

This hypothetical mechanism of degradation of linear mtDNA is presented in Scheme 2. The presence of degradation stop-sites proximal to GC-rich regions of mtDNA [37, 38] can be explained by the elevated double-strand melting temperature at these positions (GC pairing involves 3 hydrogen bonds versus 2 hydrogen bonds by AT pairing), which should slow down the helicase activity of TWNK, being also rate-limiting for replication [50]. Additionally, also the degradation speed of MGME1 is influenced by the nucleotide composition of the ssDNA fragments: GC-rich fragments show the lowest degradation speed [47]. Quite recently, direct evidence has been provided that even intrinsic oxidative stress is relevant for mtDNA turnover in HEK293 cells [8], since knock-out of LIG3 leads to accelerated mtDNA degradation due to inability of repair of intrinsic oxidative lesions.



Scheme 2. Hypothetical mechanism of mtDNA degradation after double strand breaks involving a degradation complex consisting of MGME1, POLGexo, and TWNK (modified according to the scheme presented in [37]). Importantly, binding of POLG2 to POLG is not required for the formation of the degradation complex and a contribution of other mitochondrial nucleases (EXOG, APEX2, ENDOG, FEN1, DNA2, MRE11, or RBBP8) was excluded by knock-out or knock-down experiments [37].

MITOPHAGY

As mentioned above in “Replication speed and turnover measurement of mtDNA” section, mtDNA can be also removed from cells by degradation of mitochondria as entire organelle. This process named mitophagy has been described in the literature as specific autophagic elimination of entire mitochondria. The concept of mitophagy has been originally proposed by Fletcher & Sanadi [15] as main principle to explain the concerted turnover of mitochondrial proteins and phospholipids observed in hepatocytes. In mammals, mitophagy is generally divided into PINK1/Parkin-dependent or canonical mitophagy, and PINK1/Parkin-independent mitophagy. The PINK1/Parkin-dependent pathway can be initiated by a loss of mitochondrial membrane potential [51], while PINK1/Parkin-independent mitophagy does not require loss of the mitochondrial membrane potential [52].

Table 2. Processes to be considered relevant for defining the mtDNA copy number in cells

	Speed	Enzymes/proteins involved	References
Intramitochondrial process			
Replication	2 h	POLG, POLG2, TWNK, mtSSB	[11]
Degradation of linear mtDNA	~8 h*	POLG, MGME1, TWNK	[37]
Extramitochondrial process involving singular organelles or MDVs			
Biogenesis	Synchronized with cell cycle	PGC1-α, Nrf1, Nrf2, Err-α, TFAM	[31, 32]
Mitophagy (PINK1/Parkin dependent)	4-24 h	PINK1/PARKIN	[51, 67, 68]
Mitophagy (PINK1/Parkin independent)	?	BNIP3/BNIP3L or FUNDC1 or FKBP8 or BLC2L13	[52]
Autophagy of MDVs	?	FUNDC1	[59]

Note. MDV – mitochondria-derived vesicles. * Time required for complete mtDNA degradation after induction of a double-strand break by mt*Eag*I.

PINK1/Parkin-dependent and -independent pathways utilize different sets of autophagy receptors [53]. It is assumed that the autophagy receptors involved in PINK1/Parkin-dependent mitophagy are cytosolic proteins that interact with ubiquitinated proteins of the outer mitochondrial membrane via ubiquitin-binding domains. Receptors involved in PINK1/Parkin-independent mitophagy are integral mitochondrial proteins – like BNIP3 and BNIP3L (NIX) – that interact directly with ATG8 family proteins using the general macroautophagy/autophagy machinery [53]. The latter form of mitophagy has been reported to be important for mtDNA quality control in the germline of Drosophila [54].

From a mechanistic perspective a prerequisite for mitophagy are singular organelles, thus mitochondrial fission obviously should precede degradation. Indeed, early evidence points toward mitochondrial fission being important for mitophagy [55-57], thus, supporting a direct link between mitochondrial fragmentation and mitophagy. Similarly, the axonal transport machinery for mitochondria in neurons essentially requires fragmented mitochondria as cargo [58]. However, the LIR-containing cytosolic portion of FUNDC1 is sufficient to induce mitophagy even in the absence of mitochondrial fragmentation [59], indicating that PINK1/Parkin-independent mitophagy can be uncoupled from mitochondrial morphology. Indeed, mitophagy still occurs in the absence of DRP1 in the presence of other mitophagy inducers like hypoxia, iron chelation, or proteotoxic stress [60, 61]. In such cases, a vesicle-like budding of the mitochondria has been proposed to contribute to the generation of small mitochondrial fragments that can be engulfed for the mitophagic process [62]. These mitochondrial fragments are very likely identical to the so-called MDVs described to be relevant for quality control of the cardiac system [63]. These vesicles can contain mtDNA and therefore also contribute to mtDNA degradation by the autophagic removal of leaked damaged mtDNA [64] or even entire nucleoids [65]. Therefore, the before mentioned quite large differences in the turnover times of individual mitochondrial proteins [19] and also of mtDNA (cf. “Replication speed and turnover measurement of mtDNA” section) do not exclude a substantial contribution of autophagy in turnover, since the vesicles could contain different protein and mtDNA fractions.

Studies indicate that in neurons, the sequestration of damaged mitochondria into autophagosomes can occur within approximately 1-2 h. However, the subsequent acidification and degradation within lysosomes is significantly delayed, often exceeding 6 h, and in some cases, extending beyond 24 h [66]. This suggests that the degradation phase is a rate-limiting step in neuronal mitophagy. In contrast, non-neuronal cells like HeLa cells exhibit a more rapid progression. The entire mitophagy process, from initiation to degradation, can be completed within 4-24 h, with lysosomal degradation occurring more swiftly than in neuronal cells [67, 68] (cf. Table 2). However, in this time scale mitochondrial density markers (like TRME or mitotracker fluorescence) did not change significantly [67], indicating that in these studies reporting

a fast autophagic process only very limited amounts of mitochondria were involved.

mtDNA COPY NUMBER AND DISEASE

Decreased copy numbers of mitochondrial DNA in human tissue samples are the diagnostic hallmark of so-called mitochondrial DNA maintenance disorders – a heterogeneous subgroup of mitochondrial diseases (Table 3). Here, the mtDNA copy number is defined as ratio of mtDNA copies per nucleus in the sample. Obviously, it depends on two factors: (i) the mitochondrial content of tissue and (ii) the mtDNA content of the mitochondrial network. As explained above (cf. "Determinants of mtDNA content in cells" section) the mitochondrial content of the tissue is dependent on metabolic needs, like energy requirements which affect expression of PGC-1α facilitating biosynthesis of mitochondria and on the breakdown rate of damaged mitochondria by mitophagy (cf. "Mitophagy" section). This value is obviously strongly tissue dependent with the highest values in cardiac and skeletal muscle and low values in sperm cells (cf. "Determinants of mtDNA content in cells" section). On the other hand, mtDNA is packed within nucleoids and the nucleoid content of the mitochondrial network is in the range of 300-800 nucleoids per cell [69] and each nucleoid contains about 1 copy of mtDNA [70].

Pathological deviations of cellular mtDNA content are described in following conditions:

1. Decreased mtDNA copy number due to decreased rate of replication (mutations in POLG, POLG2, TWNK, MGME1, mtSSB). This ultimately leads to low levels of mtDNA causing reduced synthesis of mtDNA-encoded proteins and strong impairment of OxPhos.
2. Decreased mtDNA copy number due to decreased rate of mtDNA replication related to nucleotide dysbalance (mutations in TK2, DGUOK, ANT1, TYMP, SUCLA2, SUCLG1, MPV17). Since dysbalance of mitochondrial desoxyribonucleotide triphosphates directly affects replication speed of POLG this causes similar to (1) lowered synthesis of mtDNA-encoded proteins and strong impairment of OxPhos. While most of the mentioned genes are directly related to the mitochondrial synthesis of phosphorylated desoxyribonucleotides, MPV17 forms an ion channel which translocates uridine but not orotate across the mitochondrial inner membrane [71].
3. Decreased mtDNA copy number due to elevated degradation of mitochondria by mitophagy (mutations in FBXL4, MFN2, OPA1). This leads to a lowered content of mitochondria and

Table 3. Mitochondrial diseases caused by alterations of the mtDNA copy number (mtDNA maintenance disorders)

Affected gene	Clinical phenotype	References
Diseases due to mutated proteins involved in replication		
POLG	muscular or brain/liver phenotype, recessive and dominant	[72, 73]
POLG2	recessive and dominant	[74]
TWNK	muscular (CPEO) or brain (ataxia) phenotype, dominant and recessive	[75]
MGME1	multisystemic phenotype, recessive	[44]
mtSSB	optic atrophy, dominant	[76]
Diseases due to mutated proteins involved in maintenance of the nucleotide pool		
TK2	muscular phenotype, recessive	[77]
DGUOK	hepatocerebral and muscular phenotype, recessive	[78]
TYMP	neurogastrointestinal encephalopathy, recessive	[79]
ANT1	cardiomyopathic phenotype, recessive and dominant	[80]
SUCLA2	encephalomyopathic phenotype, recessive	[81]
SUCLG1	encephalomyopathic phenotype, recessive	[82]
MPV17	hepatocerebral phenotype, recessive	[83]
Diseases due to mutated proteins involved in mitophagy or mitochondrial dynamics		
FBXL4	encephalomyopatic phenotype, recessive	[84]
MFN2	neuropathy of peripheral nerves, dominant and recessive	[85, 86]
OPA1	optic atrophy, dominant and recessive	[87]

consequently also of mtDNA in single cells and the tissue, however no specific OxPhos impairment can be observed. That condition affects cellular metabolism particularly in cells relying on OxPhos for energy production or the supply of precursor metabolites.

Under the conditions #1 and #2 obviously the synthesis rate of mtDNA is decreased. That leads at a constant breakdown rate of mtDNA to decreased steady state levels of mtDNA at an almost unchanged cellular content of mitochondria. As consequence a reduced transcription and translation of mtDNA-encoded proteins is observed. In contrast, condition #3 decreases the total cellular content of mitochondria due to an increase of speed of mitophagy as in case of FBXL4 mutations, which disrupt the post-translational regulation of BNIP3L and BNIP3 [88]. Similarly, mutations in proteins involved in the fusion process of mitochondrial membranes like MFN2 (outer membrane fusion) or OPA1 (inner membrane fusion) lead to a higher fragmentation of mitochondria, which also facilitates mitophagy, thus explaining reduced mtDNA copy numbers, reported in [86] and [87]. Since the reduction of mtDNA is associated under these conditions with a reduced cellular content of mitochondria, the effects on transcription and translation of mtDNA-encoded proteins are only very modest.

As can be seen in Table 3, the phenotypes detected in mtDNA maintenance disorders are as variable as reported for other mitochondrial disorders, ranging from severe encephalomyopatic to milder neuropathic or myopathic phenotypes. Genotype-phenotype correlations can be only very rarely made, since similar mutations can result, like reported for POLG mutations [72, 73], in distinct phenotypes, which is potentially related to different genetic backgrounds of the affected patients.

CONCLUSIVE REMARKS

In contrast to the nuclear genome, where almost all lesions – including DSBs – have to be repaired, the multicopy nature of mtDNA allows the degradation of severely damaged genomes, containing DSBs, to avoid deletion formation. This feature is responsible for a steady turnover of mitochondrial DNA, which is very relevant for mtDNA quality control in postmitotic cells. Therefore, cellular mtDNA maintenance and its copy number not only depend on replication speed and repair reactions. The speed of intramitochondrial mtDNA degradation performed by a POLGexo/MGME1/TWNK degradation complex and the breakdown rate of entire mitochondria (mitophagy) are also highly relevant for maintaining required steady state levels of intact mtDNA.

Abbreviations

DSB	double strand break
mtDNA	mitochondrial DNA
mtSSB	mitochondrial single-stranded DNA-binding protein
OxPhos	oxidative phosphorylation
OriH	heavy strand replication origin
OriL	light strand replication origin
SSB	single strand break

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects.

Conflict of interest

The author of this work declares that he has no conflicts of interest.

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