Mitochondrial dynamics and inheritance during cell division, development and disease

Prashant Mishra[1] and David C. Chan[1,2]

Abstract | During cell division, it is critical to properly partition functional sets of organelles to each daughter cell. The partitioning of mitochondria shares some common features with that of other organelles, particularly in the use of interactions with cytoskeletal elements to facilitate delivery to the daughter cells. However, mitochondria have unique features — including their own genome and a maternal mode of germline transmission — that place additional demands on this process. Consequently, mechanisms have evolved to regulate mitochondrial segregation during cell division, oogenesis, fertilization and tissue development, as well as to ensure the integrity of these organelles and their DNA, including fusion–fission dynamics, organelle transport, mitophagy and genetic selection of functional genomes. Defects in these processes can lead to cell and tissue pathologies.

Oxidative phosphorylation (OXPHOS). A biochemical pathway within mitochondria that generates ATP through the oxidation of nutrients.

Endosymbiotic theory
A theory postulating that genome-containing organelles, such as mitochondria and chloroplasts, are derived from bacteria that underwent endosymbiosis with ancestral eukaryotic cells.

[1]*Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, USA.*
[2]*Howard Hughes Medical Institute, California Institute of Technology, Pasadena, California 91125, USA.*
Correspondence to D.C.C.
e-mail: dchan@caltech.edu
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Organelles are a distinguishing feature of eukaryotic cells. During somatic cell proliferation, they must segregate properly to daughter cells and, during germline inheritance, a highly functional population of organelles must be transmitted to the offspring. One such organelle is the mitochondrion[1], which is best known for its critical function in energy production via oxidative phosphorylation (OXPHOS) — a pathway that generates many more ATP molecules per glucose molecule than the glycolysis pathway. Mitochondria also have important roles in other types of metabolism, in regulating intracellular calcium concentration and signalling in neurons, in the assembly of iron–sulphur clusters that are important for oxidation–reduction reactions[2], in apoptosis[3] and in innate immunity[4].

According to the endosymbiotic theory, mitochondria are descendants of ancient bacteria that entered into a symbiotic relationship with primitive host cells[5]. Mitochondria retain several characteristics of their putative bacterial ancestors: a double membrane, a proteome similar to that of α-proteobacteria and the ability to synthesize ATP via a proton gradient created across their inner membrane (BOX 1). In addition to having these bacterial characteristics, mitochondria undergo membrane remodelling through cycles of fusion (in which two mitochondria join to form a single mitochondrion) and fission (in which a single mitochondrion divides into two mitochondria)[6] (BOX 2). The balance of fusion and fission controls mitochondrial structure and, depending on the cell type, the numerous separate mitochondria in the cell can shift to form a single interconnected membranous structure.

Mitochondria have two features that constrain their segregation. First, they have a genome (mitochondrial DNA (mtDNA)), which encodes crucial bioenergetic functions (BOX 1). Each mitochondrion contains one or more mtDNA molecules, which are organized into mtDNA–protein complexes known as nucleoids. In proliferating cells, the partitioning of mitochondria to daughter cells must also result in proper distribution of mtDNA. Second, mitochondria in mammals are transmitted to subsequent generations exclusively through the maternal lineage. Because of this uniparental inheritance pattern, mtDNA lacks the benefits of recombination arising from sexual reproduction. However, mechanisms have evolved to reduce the chance of transmitting pathogenic mtDNA mutations to offspring. In some cases, these mechanisms fail, and maternally inherited disorders termed mitochondrial encephalomyopathies arise when a significant load of mtDNA mutations are passed to the offspring. These diseases are characterized by reduced mitochondrial function, with clinical signs being most pronounced in tissues with high energy consumption.

Given that mitochondria function in diverse cellular processes and that their dysfunction is implicated in several diseases, it is important to have an understanding

Genetic bottlenecks
Drastic reductions in the genetic diversity of a population.

of mitochondrial dynamics, segregation and germline inheritance. In this Review, we first outline the molecular mechanisms that ensure proper partitioning of mitochondria during cell division: the fusion–fission cycle, mitochondrial interactions with cytoskeletal elements and the endoplasmic reticulum (ER), and mitophagy. We then discuss the specific regulation of mtDNA inheritance and its uniparental transmission through the maternal lineage, including the evidence for genetic bottlenecks during oogenesis and embryonic development, and the mechanisms preventing transmission of paternal mitochondria to offspring. Throughout, our main focus is on mammalian mitochondria, but studies carried out in yeast, worms and flies are discussed in cases in which they have provided substantial insights.

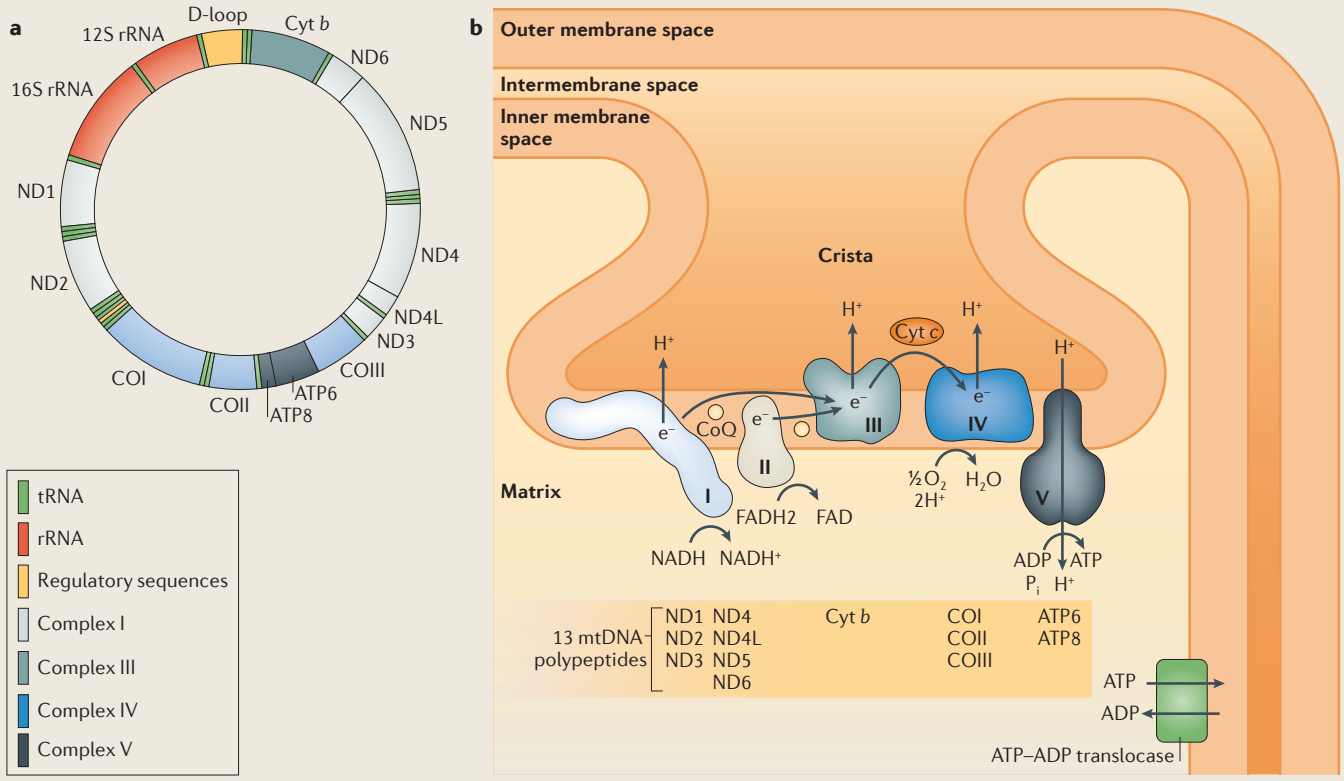
Mitochondrial dynamics and segregation

In discussing mitochondrial inheritance, 'proper partitioning' of the mitochondria implies that mitochondria and mtDNA are distributed equally to daughter cells (in the case of symmetric cell division), and that the inherited mitochondria are highly functional. In mammalian cells that divide symmetrically, segregation could, in principle, be a largely passive process, as long as there are mechanisms to ensure a homogeneous and evenly dispersed spatial distribution of organelles throughout the cell soma (FIG. 1). As discussed below, faithful segregation is facilitated by the induction of mitochondrial fission during the G2 and mitosis phases of the cell cycle (FIG. 1b) and by the interaction of mitochondria with cytoskeletal and ER elements, which are spread throughout the cell

Box 1 | The mitochondrial genome and oxidative phosphorylation

Mitochondria contain a residual genome (mitochondrial DNA (mtDNA)), which is critical for their function in oxidative phosphorylation (OXPHOS). In humans, the mtDNA is ~16.6 kilobases in length and contains 37 genes encoding 13 polypeptides, 2 ribosomal RNAs and 22 tRNAs (see the figure, part **a**). The displacement loop (D-loop) is a regulatory sequence that controls mtDNA replication and transcription. The 13 polypeptides are distributed among the OXPHOS protein complexes I, III, IV and V and are essential for OXPHOS (cellular respiration) activity (see the figure, part **b**). The specific mtDNA-encoded subunits are listed under each respiratory chain complex. These complexes also have multiple subunits encoded by the nuclear genome. Complex II is encoded entirely by the nuclear genome. The 2 rRNAs and 22 tRNAs encoded by the mtDNA are essential for the mitochondrial translational machinery that is used to generate the 13 polypeptides and, because of this, all 37 mtDNA genes are critical for OXPHOS. The remaining proteins (>1,000) in the mitochondrial proteome are encoded by the nuclear genome, synthesized in the cytosol and imported into the mitochondria.

Mitochondria are double-membrane organelles consisting of an outer membrane surrounding an inner membrane of greater surface area. The space between the two membranes is termed the intermembrane space. Because of its greater surface area, the inner membrane folds back on itself, creating compartments termed cristae. The respiratory chain complexes are concentrated in the cristae membranes. Complexes I, III and IV pump protons from the matrix (the compartment enclosed by the inner membrane) to the intermembrane space, generating an electrochemical gradient across the inner membrane that is used to power ATP production by complex V (ATP synthase). The ATP–ADP translocase exports the ATP into the intermembrane space, where it freely diffuses out of the outer membrane into the cytosol. The mtDNA is packaged into nucleoid structures (not shown) located in the matrix.

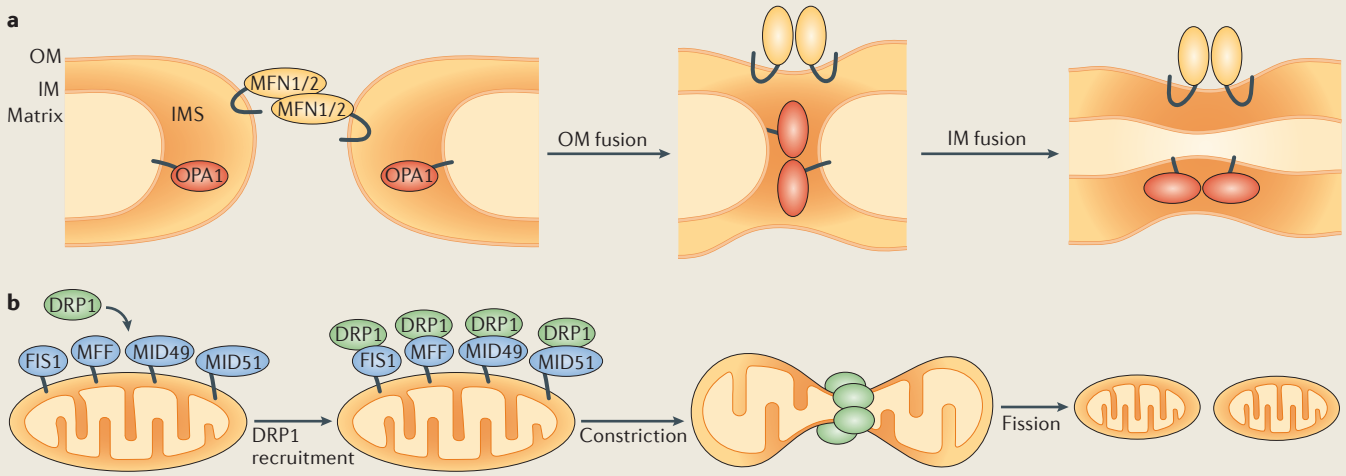


ATP6, ATP synthase 6; CO, Cyt *c* oxidase subunit; CoQ, Coenzyme Q_{10}; Cyt, cytochrome; ND, NADH–ubiquinone oxidoreductase chain; P_i, inorganic phosphate.

Box 2 | **Mitochondrial fusion and fission in mammalian cells**

Continual cycles of fusion and fission result in the intermixing of the mitochondrial population in the cell[6]. These opposing processes also determine mitochondrial morphology. Increased fusion or reduced fission promotes the formation of elongated mitochondrial networks, whereas increased fission or reduced fusion causes mitochondrial fragmentation (FIG. 1a). The key factors in both fusion and fission are large GTP-hydrolysing enzymes of the dynamin superfamily. Mitochondrial fusion consists of two sequential steps: first, the outer membranes (OMs) of two mitochondria fuse; second, the inner membranes (IMs) fuse (see the figure, part **a**). OM fusion is mediated by mitofusin 1 (MFN1) and MFN2, which are dynamin-related GTPases at the OM. IM fusion is mediated by the dynamin-related protein optic atrophy 1 (OPA1). OPA1 exists in two forms: a long form, which is integral in the IM, and a short form (not shown), which is targeted to the intermembrane space. Whereas cells lacking both MFNs have no mitochondrial OM fusion, cells lacking OPA1 do undergo mitochondrial OM fusion events, but the fusion intermediates cannot progress to IM fusion[102,103].

The opposing process of mitochondrial fission (see the figure, part **b**) requires the recruitment of dynamin-related protein 1 (DRP1) from the cytosol to the mitochondrial OM. Assembly of DRP1 on the mitochondrial surface causes constriction of the mitochondria and eventual division of the organelle into two separate entities. Four DRP1 receptors exist in mammals — mitochondrial fission 1 (FIS1), mitochondrial fission factor (MFF), Mitochondrial dynamics protein of 49 kDa (MID49) and MID51 — and these are located on the mitochondrial OM. MFF, MID49 and MID51 have prominent roles in fission; however, despite its importance in yeast, FIS1 seems to play only a minor part in mitochondrial fission in mammals[104,105].



soma. Active mechanisms that deliver mitochondria to daughter cells are not known in mammalian cells; however, this may reflect a lack of suitable model systems in which to study this process. In particular, little is known about mitochondrial segregation in mammalian cells that divide asymmetrically. In budding yeast cells, which undergo asymmetric cell division, mitochondrial segregation is driven by mechanisms that actively deliver a subset of mitochondria into the daughter cell while retaining another subset in the mother cell (BOX 3).

Microtubule-based mitochondrial motility. Mitochondrial distribution within the cell soma depends on mitochondrial trafficking along the cytoskeleton. In mammals, microtubule filaments, which are created by the polymerization of α-tubulin and β-tubulin subunits, form a cytoskeletal platform for the distribution and transport of mitochondria. The colocalization of mitochondria and microtubules has been well documented, and treatment of cells with microtubule-depolymerizing agents results in misalignment and redistribution of mitochondria[7,8]. Depending on the cellular context, actin and intermediate filaments can also regulate mitochondrial distribution. For example, the actin cytoskeleton plays a prominent part in organizing the presynaptic terminal and dendritic spines of neurons, and actin filaments help to recruit mitochondria to these regions[9].

Motor proteins transport mitochondria along microtubules: kinesins transport mitochondria in the plus-end (anterograde) direction, and dyneins transport mitochondria in the minus-end (retrograde) direction. Numerous kinesin family members have been implicated in mitochondrial anterograde transport. For instance, deletion of KIF5B, a kinesin-1 family member, in mammalian placental cells leads to a dramatic redistribution of mitochondria — rather than being evenly distributed throughout the cell, they accumulate into a single perinuclear cluster[10]. This observation suggests that kinesin-based motility in the microtubule plus-end direction is required for proper mitochondrial distribution throughout the cell body. Other studies have provided evidence for the involvement of kinesins in mitochondrial anterograde motility in mammalian neurons[11–13]. In contrast to yeast cells (BOX 3), deficiencies in mitochondrial motor proteins have not yet been causally linked with mitochondrial segregation defects in mammalian cells.

Additional components of the kinesin-based transport system have been identified in genetic screens of *Drosophila melanogaster* neuronal function[14]. The carboxy-terminal cargo-binding domain of Kinesin-1 interacts with an adaptor protein called Milton, which in turn binds to Mitochondrial Rho GTPase (Miro), an integral membrane protein localized to the mitochondrial outer membrane (FIG. 1c). In fly neurons, anterograde mitochondrial transport is severely impaired in

Motor proteins
Molecular motors that use ATP hydrolysis to power the movement of cargo along substrate surfaces.

Kinesins
A family of ATP-dependent molecular motors that transport cargo along microtubule filaments, usually towards the plus end.

Dyneins
A family of ATP-dependent molecular motors that transport cargo along microtubule filaments towards the minus end.

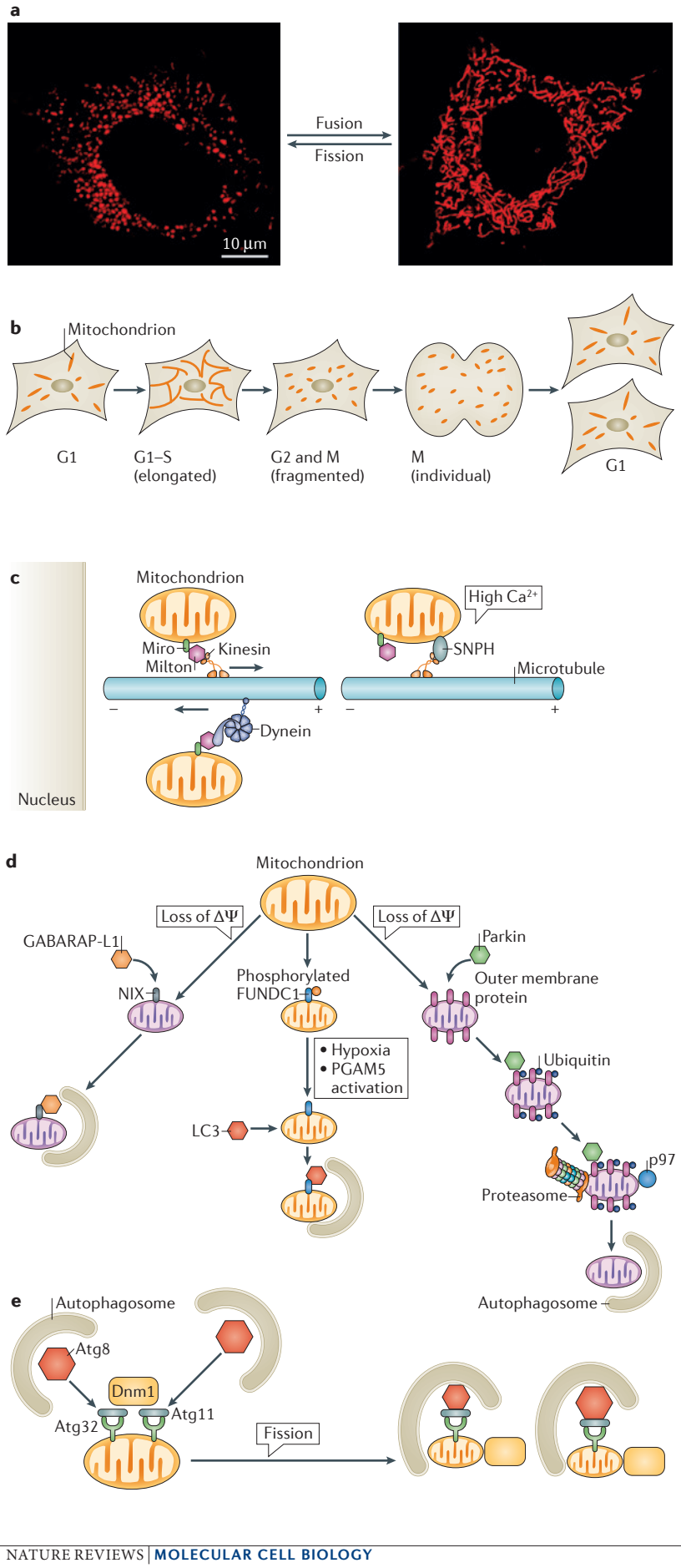
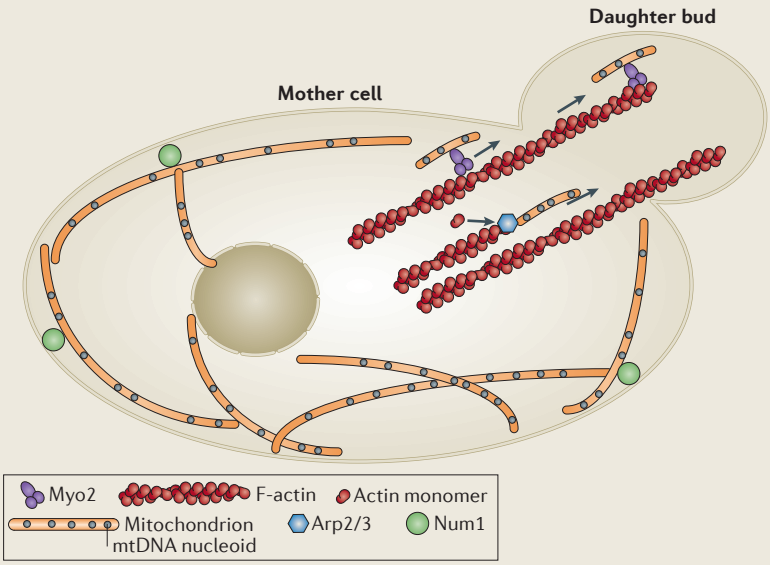


Figure 1 | **Cellular mechanisms involved in mitochondrial segregation, transport and degradation.** **a** | The control of mitochondrial morphology by fusion and fission. Cells with increased fission or reduced fusion have small mitochondria (left), whereas cells with increased fusion or reduced fission have elongated mitochondrial tubules (right). Mitochondria were visualized in mouse embryonic fibroblasts by expression of mitochondrially targeted DsRed. **b** | Cell cycle mitochondrial dynamics. Mitochondrial morphology is coordinated with the cell cycle and promotes equal segregation of mitochondria during cell division. At the G1 stage of the cell cycle, mitochondria have various morphologies. During the G1–S transition, mitochondria fuse and elongate, presumably in preparation for the high metabolic demand associated with genome replication. By contrast, at the G2 and M phases, mitochondria undergo fission and form numerous individual organelles that are spatially distributed throughout the soma. This enables the equal distribution of mitochondria to each of the daughter cells during mitosis. Upon re-entry to the G1 phase, mitochondria regain some of their elongated structure. **c** | Mitochondrial transport. Mitochondria can associate with cytoskeletal filaments and be transported along them by molecular motors. Mitochondria in mammalian cells are transported mostly on microtubule filaments by kinesin and dynein motors[14]. Kinesins transport mitochondria towards the plus end of microtubules, whereas dyneins transport them towards the minus end (left). Milton acts as an adaptor to link each motor to Mitochondrial Rho GTPase (Miro), which is on the mitochondrial surface. In the presence of high calcium concentrations (right), kinesin is released from the Milton–Miro complex and instead binds to syntaphilin (SNPH). SNPH inhibits the ATPase activity of kinesin, and this results in the immobilization of mitochondria. **d** | Mitophagy pathways in mammalian cells. Parkin (right) is recruited to dysfunctional mitochondria (purple) with reduced membrane potential ($\Delta\Psi$)[45]. Parkin is an E3 ubiquitin ligase and it polyubiquitylates a large number of mitochondrial outer membrane proteins, resulting in their degradation by the ubiquitin–proteasome system[47,48], which is necessary for the subsequent removal of mitochondria from the cytosol[47]. The AAA (ATPases associated with various cellular activities) ATPase p97 facilitates the degradation of outer membrane proteins by the 26S proteasome. In erythroid cells (left), removal of mitochondria requires the outer membrane protein NIP3-like X (NIX), which interacts with the light chain 3 (LC3) homologue GABA (γ-aminobutyric acid) receptor-associated protein L1 (GABARAP-L1). In hypoxia-induced mitophagy (middle), the phosphatase phosphoglycerate mutase family member 5 (PGAM5) dephosphorylates the LC3-interacting region of FUN14 domain-containing 1 (FUNDC1). Dephosphorylated FUNDC1 then recruits LC3 to bring autophagosomes to the mitochondria. **e** | Mitophagy in yeast. Mitophagy is induced during post-log growth in non-fermentable media. Such conditions induce the expression of autophagy-related 32 (Atg32), which acts as a receptor to recruit the autophagy machinery[62,63]. Atg32 binds to Atg8 (the yeast orthologue of LC3), thereby linking the mitochondrion to autophagosomes. Atg32 also binds to Atg11, which interacts with dynamin-related 1 (Dnm1)[64] (BOXES 2,4), a central component of the mitochondrial fission machinery, to promote mitochondrial fission during mitophagy.

Box 3 | **Active segregation of mitochondria in yeast**



The budding yeast *Saccharomyces cerevisiae* is an attractive model system to study the mechanisms controlling mitochondrial cytoplasmic distribution and segregation. In this organism, cell division is a highly polarized process whereby a daughter bud forms at a distinct site on the mother cell's surface. Mitochondria and other organelles are rapidly transported to the emerging bud, which eventually divides off as the daughter cell. In fact, the number of mitochondria increases in direct relation to the steadily increasing volume of the bud until a specific ratio of mitochondrial to bud volume is met[106]. This suggests that the mitochondrial content in the bud is carefully monitored to ensure that sufficient mitochondria are distributed to the daughter cell, even if it compromises the mitochondrial content of the ageing mother cell[106]. Yeast genetic screens tracking the delivery of mitochondria during budding have identified factors that are important to mitochondrial transport to the growing bud[107].

The actin cytoskeleton actively transports mitochondria into the bud (see the figure). During bud emergence, formin proteins (not shown) are deposited at the future bud site and direct polymerization of F-actin, linking the mother cell to the bud[108]. These mother-to-bud actin filaments serve as 'tracks' to deliver mitochondria and other organelles to the daughter cell[109–111]. Because actin-related protein 2/3 (Arp2/3) mutants have mitochondrial motility defects, it has been proposed that Arp2/3 nucleates a burst of actin polymerization on the mitochondrial surface that drives transport along the mother-to-bud actin filaments[112,113] (see the figure). This model has similarities to the transport mechanism used by some mobile intracellular bacteria[114]. However, subsequent studies have supported an alternative model, in which myosin 2 (Myo2) is a motor protein with a direct role in transporting mitochondria as cargo into the bud[115–117] (see the figure). Myo2 is used for transport of most membrane-bound organelles in budding yeast[118–123]. The Myo2 receptor on mitochondria remains to be defined, although candidates include Mitochondrial MYO2 receptor-related 1 (Mmr1) and Ypt11, which physically interact with Myo2; indeed, yeast cells that are deficient in both *mmr1* and *ypt11* fail to transport mitochondria into the bud[116,124,125]. Myosin molecules may also have a role in organelle delivery during other asymmetric cell divisions; for instance, Myo6 has been implicated in active mitochondrial delivery during spermatogenesis in *Caenorhabditis elegans*[126].

The delivery of mitochondria to the daughter bud is balanced by retention of mitochondria in the mother cell. Recent genetic and biochemical studies indicate that the cortical protein nuclear migration 1 (Num1) serves as a plasma membrane anchor that interacts with mitochondria and retains them in the mother cell[127,128] (see the figure).

Formin proteins
A group of proteins that regulate the actin cytoskeleton and cell signalling.

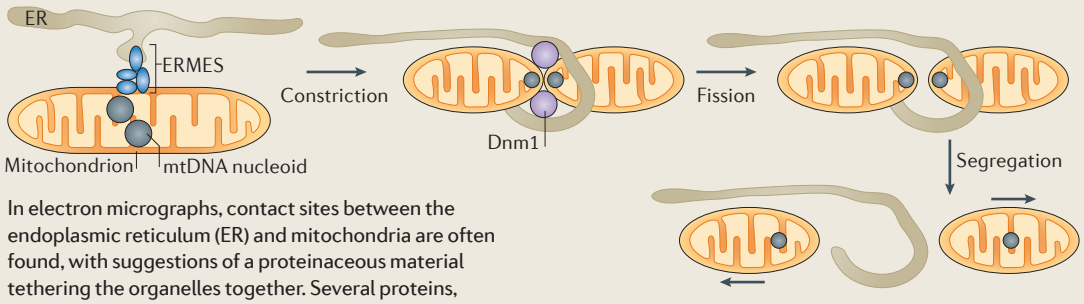
the absence of either Milton or Miro[15,16]. Interestingly, Miro has calcium-binding EF-hand domains, which are involved in stalling mitochondria in response to local elevation of calcium concentration at active synapses within axons[17]. In mouse axons, an additional important factor that regulates mitochondrial localization is local stalling of mitochondria — at any given time, most mitochondria in axons are immobile. This pausing results from anchoring of axonal mitochondria to the microtubule cytoskeleton by syntaphilin (SNPH)[18]. When calcium levels are high, Miro releases kinesin-1, which is then bound and inhibited by SNPH[19] (FIG. 1c). This close interplay between SNPH and the Milton–Miro transport complex may explain why axonal mitochondria often show periods of pausing during active transport. Moreover, data from proliferating mammalian cells suggest that the mitochondrial kinesin complex has an important role in regulating the mitochondrial distribution throughout the cell soma. In mammalian fibroblasts, co-overexpression of Milton and KIF5B results in clustering of mitochondria at the cell periphery[20], and KIF5B deletion results in mitochondrial accumulation around the nucleus. Thus, the Miro–Milton–KIF5B complex can regulate mitochondrial distribution through the promotion of anterograde motility. Recent data suggest that Miro and Milton can also regulate retrograde motility through their association with dynein motor proteins[21] (FIG. 1c).

Because cells lacking Miro or Milton function show dramatic changes in mitochondrial distribution, it is possible that these molecules play a part in mitochondrial partitioning during cell division. However, this point remains to be experimentally addressed, because most studies have used non-dividing neurons. In contrast to the situation in mammals, mitochondrial motility and distribution in budding yeast are dependent on the actin cytoskeleton, and mutants in these processes show clear segregation defects (BOX 3).

Fusion and fission ensure mitochondrial homogenization. During mitochondrial fusion, two mitochondria merge their outer and inner membranes, resulting in a single, larger mitochondrion. Mitochondrial fission is the opposite process, whereby a mitochondrion divides into two organelles. Fusion of mitochondria is mediated by three dynamin-related GTPases — mitofusin 1 (MFN1) and MFN2 on the mitochondrial outer membrane and optic atrophy 1 (OPA1) on the inner membrane — whereas fission is mediated by dynamin-related protein 1 (DRP1; also known as DNM1L)[6], which is also a large GTPase (BOX 2). Complete deficiencies in any of these proteins is embryonically lethal in mice, and mutations in some of these proteins are associated with neuromuscular disease in humans[6,22]. Studies in cell culture and tissues suggest that fusion and fission are critical for maintaining the health of the mitochondrial population[23–26]. For example, when mitochondrial fusion is blocked, Purkinje neurons in the cerebellum degenerate owing to defects in electron transport and mtDNA maintenance[27].

How could fusion and fission maintain the proper function of mitochondria? Cells lacking the MFNs or OPA1 show a striking heterogeneity among mitochondria in protein content, mtDNA nucleoid content and membrane potential[23,27–29]. As a result, mutant cells show defects in respiratory chain function[28] and accumulate mtDNA deletions[23]. Thus, cycles of fusion and fission promote mixing of mitochondrial content and homogenization of mitochondrial protein and DNA in the cell.

Box 4 | Endoplasmic reticulum–mitochondria connections



In electron micrographs, contact sites between the endoplasmic reticulum (ER) and mitochondria are often found, with suggestions of a proteinaceous material tethering the organelles together. Several proteins, including mitofusin 2 (MFN2)[129], have been implicated in the formation of these close contacts, which are important for transmission of calcium signals from the ER to the mitochondria[130] and for phospholipid biosynthesis[131,132]. In addition, ER tubules seem to initiate mitochondrial fission in both yeast and mammals by wrapping around and constricting the organelle[133]. This ER–mitochondria contact is an early event observed in many, but not all, fission events. It occurs before recruitment of the fission factor dynamin-related 1 (Dnm1; known as dynamin-related protein 1 (DRP1) in mammals)[133], which further constricts the mitochondrial tubule to mediate scission. The actin cytoskeleton has also been shown to be important for mitochondrial fission[134].

Components of the ER–mitochondria tether, termed the ERMES (ER–mitochondria encounter structure) complex, have been identified in budding yeast[135]. The complex consists of the mitochondrial outer membrane proteins mitochondrial distribution and morphology 10 (Mdm10) and Mdm34, the integral ER membrane protein maintenance of mitochondrial morphology 1 (Mmm1), and the peripheral mitochondrial outer membrane protein Mdm12. Because of its role in mediating ER–mitochondrial contacts, ERMES may have a significant role in regulating yeast mitochondrial fission. Recent results further suggest that ERMES may have a role in linking mitochondrial fission to segregation of mitochondrial DNA (mtDNA) nucleoids[136]. Components of the ERMES complex are located adjacent to replicating mtDNA nucleoids and may facilitate the equal distribution of mtDNA nucleoids between daughter mitochondria during the fission event[136] (see the figure). Perhaps owing to these roles in mitochondrial fission, the ERMES components Mdm10, Mdm12 and Mdm34 were originally isolated in yeast screens for genes required for mitochondrial transport to the daughter bud[107,137,138] (BOX 3). No orthologues of the ERMES complex have been found in mammals.

Myosin
A family of ATP-dependent molecular motors that transport cargo along actin filaments.

Dynamin-related GTPases
A family of large GTP-hydrolysing enzymes related to 'classical' dynamin, which use GTP hydrolysis to mechanically remodel membranes.

mtDNA nucleoid
A punctate structure within mitochondria that contains one or more copies of the mitochondrial DNA (mtDNA) genome in complex with proteins.

Macroautophagy
The most commonly studied autophagic process, whereby cellular components are incorporated into autophagosomes and ultimately degraded or recycled through the lysosome. Macroautophagy is commonly referred to as simply 'autophagy'.

Autophagosomes
Double-membrane structures that engulf cellular components for delivery to lysosomes.

Lysosomes
Organelles that degrade cellular components using acid hydrolases.

Functional mitochondria continually mix their contents, and quality-control mechanisms exist to prevent grossly dysfunctional mitochondria from fusing with healthy mitochondria[30]. For example, mitochondria that lose their inner membrane potential undergo proteolytic inactivation of OPA1 (REF. 31), resulting in loss of inner membrane fusion, which prevents their reincorporation into the cellular mitochondrial population network[30]. In addition, many forms of mitochondrial dysfunction result in activation of mitochondrial fission or inactivation of fusion[31,32]. These dynamic changes promote the conversion of dysfunctional mitochondria into smaller physical units, which can be targeted for degradation via macroautophagy (see below). Conversely, mitochondria that elongate owing to inhibition of the fission activity of DRP1 are protected from autophagy, presumably because of their enlarged size[33,34].

Mitochondrial fusion and fission dynamics are regulated during the cell cycle[35]. Enhanced fusion is associated with the G1-to-S phase transition, when the mitochondria tend to look like large, interconnected networks (FIG. 1b). Mitochondrial fission is activated during mitosis[35,36] through the phosphorylation of DRP1, resulting in discrete mitochondrial units. Because they are associated with cytoskeletal elements and with the ER (BOX 4), these individual mitochondria are distributed evenly throughout the cell soma, which ensures that they are passively partitioned in equal numbers to daughter cells during cytokinesis. In cells lacking DRP1, the mitochondria exist constitutively as elongated networks owing to the lack of mitochondrial fission. Nevertheless, mitochondria are segregated to daughter cells, presumably because the cytokinesis machinery is sufficiently strong to forcibly cleave mitochondria[25,26]. However, the partitioning of mitochondria to daughter cells in DRP1-mutant cells seems to be less uniform, perhaps because the mitochondria remain in large networks during mitosis[25], although this observation remains to be quantified systematically. It is unclear whether this segregation defect has any physiological consequence in the daughter cells.

So, the combined effects of fusion and fission promote a homogeneous and healthy population by mixing mitochondrial protein and DNA contents throughout the mitochondrial population of the cell, and enhanced fission at the onset of mitosis facilitates equal passive segregation mitochondria to daughter cells.

Mitochondrial clearance by mitophagy

Mitophagy is the sequestration of mitochondria by autophagosomes followed by their degradation in lysosomes. Thought to be the primary mechanism for degradation of mitochondria, mitophagy controls the composition of the mitochondrial population in the cell by culling subpopulations of mitochondria that are dysfunctional. Although mitochondria can be non-selectively removed as part of a bulk autophagy response, mitophagy can be selective for damaged mitochondria under certain cellular contexts. For instance, mitochondria that are experimentally depolarized attract the autophagy machinery and undergo mitophagy[37,38]. In yeast, nitrogen-starvation leads to bulk cellular autophagy that includes mitophagy. However, if cells starved for nitrogen are kept

on a carbon source requiring mitochondrial metabolism, mitophagy (but not cellular autophagy in general) is reduced, indicating that mitophagy can be differentially regulated from bulk autophagy[39]. By selectively targeting dysfunctional mitochondria, mitophagy could maintain the quality of the mitochondrial population. However, how efficient mitophagy is in achieving this remains unclear, as there are many pathological states, such as mitochondrial encephalomyopathies, in which highly dysfunctional mitochondria or pathogenic mtDNA mutations persist in cells and tissues[40].

The PINK1–parkin mitophagy pathway. The most studied mitophagy pathway involves PTEN-induced putative kinase 1 (PINK1) and parkin; mutations in their encoding genes are involved in familial cases of Parkinson's disease[41]. As first shown in fly mutants, Parkin acts downstream of PINK1 to maintain mitochondrial function[42–44]. In mammalian cell culture models, these two proteins promote mitophagy of compromised mitochondria. In response to depolarization of the mitochondrial inner membrane, parkin (which is an E3 ubiquitin ligase), translocates from the cytosol onto the mitochondrial outer membrane in a PINK1-dependent manner[45,46], where it causes widespread polyubiquitylation of mitochondrial outer membrane proteins[47,48]. This polyubiquitylation promotes degradation of mitochondrial outer membrane proteins by the 26S proteasome[47,49]. Protein degradation is also dependent on the AAA (ATPases associated with various cellular activities) ATPase p97 (REF. 50), which may have a role in extracting proteins from the mitochondrial outer membrane to make them more accessible to the proteasome[51]. Degradation of mitochondrial outer membrane proteins is necessary for the mitophagic response[47] (FIG. 1d). Overexpression of parkin in cytoplasmic hybrid (cybrid) cells, which are heteroplasmic for a mutation in the mitochondrial cyclooxygenase 1 gene, reduces the level of the pathogenic mutation, suggesting that the PINK1–parkin pathway can introduce bias in the maintenance of mtDNA genotypes[52]. The functions of PINK1 and parkin in mitophagy have mostly been studied by overexpression in mammalian cell lines. However, their involvement in mitophagy is supported by proteomic analyses, indicating that flies mutated in these genes have slower turnover of mitochondrial proteins[53].

Other mitophagy pathways. During erythrocyte maturation, intracellular organelles such as mitochondria are completely degraded. It has been suggested that the removal of damaged mitochondria is important in preventing downstream pathways that lead to erythrocyte cell death[54]. The mitochondria are eliminated by sequestration into autophagosomes, but the process may involve both canonical and non-canonical autophagy pathways. At least some mitochondrial degradation persists in the absence of core components of the autophagic machinery, such as autophagy-related 5 (ATG5)[55] or ATG7 (REF. 56). The BCL-2 homology 3 (BH3)-containing protein NIP3-like X (NIX; also known as BNIP3L), which is located on the mitochondrial outer membrane, has been shown to be important for the elimination of mitochondria from erythrocytes (FIG. 1d). In *Nix*-knockout mice, approximately one-third to half of the circulating erythrocytes show aberrant persistence of mitochondria, even though other cellular components, such as ribosomes, are degraded[57,58]. During maturation, erythroid mitochondria normally show loss of membrane potential, which probably acts as a signal to promote mitophagy. Mitochondrial membrane depolarization fails to occur in NIX-deficient erythroid cells, and artificial dissipation of the membrane potential can restore mitophagy[58]. NIX contains an amino-terminal LC3-interacting region (LIR), which binds to light chain 3 (LC3; also known as MAP1LC3B)-like proteins — especially GABA (γ-aminobutyric acid) receptor-associated protein L1 (GABARAP-L1)[59] —which are ubiquitin-related proteins that are located on autophagosomes and are important for their maturation. The LIR is essential for the ability of NIX to mediate mitophagy[59]. These observations suggest that NIX is a selective mitophagy receptor that physically connects the autophagy machinery to the mitochondrial surface in erythroid cells.

In cultured mammalian cells, another mitophagy receptor is the outer membrane protein FUN14 domain-containing 1 (FUNDC1), which regulates autophagic degradation of mitochondria in response to hypoxia[60] (FIG. 1d). Overexpression of FUNDC1 reduces total mitochondrial mass in cells, and FUNDC1 depletion conserves mitochondrial mass during hypoxia[60]. FUNDC1 has a LIR, which is important for recruitment of LC3 and for mitochondrial degradation. Under hypoxic conditions, the mitochondrial phosphatase phosphoglycerate mutase family member 5 (PGAM5) dephosphorylates the LIR, which increases its physical association with LC3 and consequently promotes mitophagy[61].

In yeast, genetic screens have identified the outer membrane protein Atg32 as a mitophagy receptor[62,63] (FIG. 1e). Atg32 contains an LIR that binds to Atg8, the yeast LC3 orthologue, which is located on the surface of autophagosomes. In addition, it binds to Atg11, an adaptor for several selective forms of autophagy. Atg11 has been shown to interact with dynamin-related 1 (Dnm1)[64], the yeast orthologue of DRP1, which is involved in mitochondrial fission. On the basis of these observations, it has been suggested that, when Atg11 has been recruited by Atg32 to mitochondria, Dnm1 is recruited to these marked mitochondria to facilitate fission[64], and Atg8 is recruited to target them to autophagosomes. The process of fission may be important for generating smaller mitochondrial fragments that can be segregated into autophagosomes. Mitochondrial fission has also been shown to facilitate parkin-mediated mitophagy[50]. Nevertheless, a study in yeast indicates that Dnm1 and other components of the mitochondrial fission machinery are dispensable for mitophagy[65].

Taken together, these results suggest that mitochondrial degradation depends on specific mitophagy receptors present on the mitochondrial outer membrane that function to target mitochondria to the autophagic machinery. It is also possible that, in some cases, ubiquitylation serves to mark mitochondria for autophagy. In addition, mitophagy can involve outer membrane

Cytoplasmic hybrid (cybrid) cells
Hybrid cells created by the fusion of a cell with an enucleated second cell, thereby combining the nucleus and mitochondria from two distinct cells.

Heteroplasmic
A state in which more than one haplotype of mitochondrial DNA exists in a cell or an organism.

protein degradation by the ubiquitin–proteasome system and the AAA ATPase p97, and reduction of mitochondrial size by mitochondrial fission[47,50]. By culling damaged mitochondria, particularly mitochondria with defective mtDNA, mitophagy has the potential to regulate the functionality of the mitochondrial populations inherited by daughter cells; however, more work is necessary to understand the physiological effect of this process.

mtDNA germline dynamics and inheritance

A typical human cell contains hundreds of copies of mtDNA, and until a few years ago it was assumed that individuals exhibit homoplasmy for a single mtDNA genotype. This assumption has certain consequences, for example, when analysing mtDNA genotypes in forensic cases and for tracing human lineages during evolution. Significant heteroplasmy was known to occur in patients with mitochondrial encephalomyopathies (see below), but these cases were thought to be the exception rather than the rule. However, with the advent of deep sequencing, it has become clear that low-level heteroplasmy in humans is normally present in most tissues[66,67]. The source of this heteroplasmy is twofold. A portion is maternally inherited, as evidenced by the observation that some of the low-frequency alleles found in an individual can be found also in the mother. Another portion, not present in the mother, is presumed to arise from *de novo* mutations that occur during tissue development[66,67].

Because the mtDNA encodes respiratory chain proteins, as well as the tRNAs and ribosomal RNAs that are necessary for their translation (BOX 1), mutations in the mitochondrial genome result in OXPHOS defects that cause a large collection of diseases termed encephalomyopathies. These diseases have a surprisingly broad range of clinical phenotypes, but tissues that are highly metabolically active, such as skeletal muscle, heart muscle, nerve and brain, tend to be the most commonly affected[40]. Encephalomyopathies can be familial or sporadic. When familial, they have a maternal inheritance pattern because mtDNA is passed from the mother to her offspring. The most common familial encephalomyopathy is Leber's hereditary optic neuropathy (LHON), which is caused by complex I deficiency owing to mutations predominantly in the NADH–ubiquinone oxidoreductase chain 1 (*ND1*), *ND4* or *ND6* genes of the mitochondrial genome (BOX 1). LHON typically causes bilateral deterioration of central vision through the loss of retinal ganglion cells and atrophy of the optic nerve[68,69]. In sporadic encephalomyopathies, respiratory chain defects originate from a *de novo* mutation. For example, in chronic progressive external ophthalmoplegia (CPEO), mtDNA genomes containing a partial deletion are found only in muscle tissue, implying that the mutation arose in precursor cells of this lineage[40]. CPEO causes weakness in the eye-associated muscles, resulting in inability to raise the eyelids (ptosis) and difficulty in moving the eyeballs (ophthalmoplegia).

Understanding mtDNA segregation and inheritance has biomedical implications for discerning how mutations in mtDNA can cause the wide variability and diversity of tissue defects found in mitochondrial encephalomyopathies. Such diseases often have a progressive and variable clinical outcome. Patients inherit a burden of pathogenic mtDNA from their mothers, but the levels of such mtDNA are typically not high enough to cause an immediate OXPHOS deficiency in cells. As an individual ages, genetic drift during cell proliferation results in variability in the levels of pathogenic mtDNA between cells. Only cells or tissues that acquire very high levels of pathogenic mtDNA will exhibit an OXPHOS deficiency. The threshold for this bioenergetic breakdown is fairly high: most mtDNA mutations need to accumulate to >60–90% of total mtDNA in the cell before OXPHOS activity is compromised[70–72]. In both mouse[73] and human cells[74], the complementation of mtDNA mutations was proposed to depend on mixing of genomes by mitochondrial fusion. In a mouse that was genetically engineered to have high mtDNA mutation rates, reduction in mitochondrial fusion indeed exacerbated the effects of mtDNA mutations[23].

mtDNA undergoes a genetic bottleneck during oogenesis. Analysis of mtDNA transmission in animal pedigrees has provided evidence that heteroplasmic mtDNA genotypes can rapidly segregate in offspring. This phenomenon has been particularly well-documented in Holstein cows[75], in which the offspring from a heteroplasmic mother can have mtDNA haplotype ratios that differ significantly from each other and/or from the mother. In extreme cases, a mother with low levels of heteroplasmy can give rise to offspring that are homoplasmic for the rare haplotype[75]. These observations support the hypothesis that a severe mtDNA genetic bottleneck occurs during oogenesis, such that only a small fraction of mtDNA molecules are ultimately represented in the mature egg (FIG. 2). Experimental and theoretical analyses suggest that this bottleneck in mice consists of several hundred segregating molecules of mtDNA[76]. A similar situation occurs in humans and has implications on our understanding of familial mitochondrial encephalomyopathies, as clinical studies indicate that a heteroplasmic mother can have offspring whose pathogenic mtDNA content varies substantially from her own[77,78].

Although the occurrence of a mtDNA bottleneck during oogenesis is widely accepted, its molecular mechanism is unclear, as recent studies have provided conflicting data. During oocyte maturation, a dramatic increase occurs in mitochondrial content and mtDNA copy number, culminating in mature (metaphase II) oocytes containing >100,000 copies of mtDNA, presumably in preparation for the metabolic demands of fertilization and implantation. The mtDNA bottleneck is thought to occur before this period of rapid mtDNA accumulation. Several mechanisms could potentially lead to rapid segregation of mtDNA haplotypes. The bottleneck may be numerical; that is, the level of mtDNA might be transiently constricted earlier in oogenesis, thereby limiting the diversity of mtDNA molecules that will be present in the mature oocyte. In this case, rapid segregation of haplotypes between oocytes would be achieved by the reduction of the number of mtDNA molecules present in each oocyte and by random genetic drift. Quantitative measurements of mtDNA numbers during oogenesis provide evidence for[79] and against[80,81] the severe developmental contraction

Homoplasmy
A state in which a single haplotype of mitochondrial DNA exists in a cell or an organism.

Genetic drift
A change in the allele or haplotype frequencies within a population owing to stochastic forces.

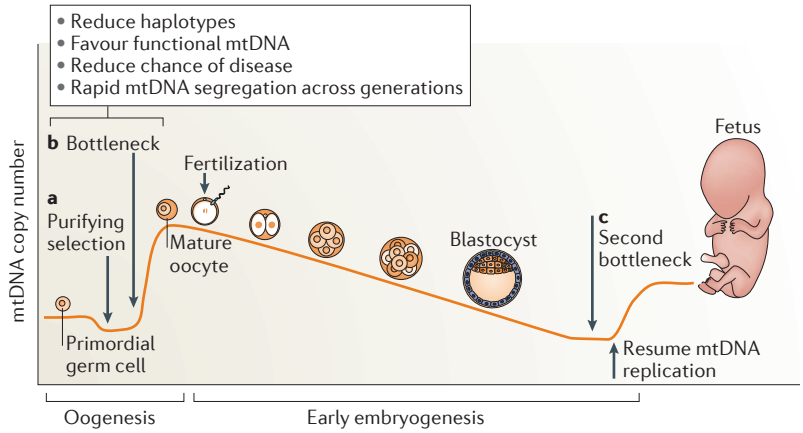


Figure 2 | **mtDNA segregation during maternal transmission and early embryogenesis.** Two major mechanisms affect mitochondrial DNA (mtDNA) genotypes during oocyte development: purifying selection (part **a**) and the mtDNA genetic bottleneck (part **b**). These are followed by a second bottleneck in early embryogenesis (part **c**). By eliminating oocytes containing deleterious mtDNA mutations, purifying selection reduces the possibility of severe mtDNA disease and favours the preservation of functional mtDNA. Little is known about the exact timing of this purifying selection, but one study suggests that it occurs during oogenesis before the genetic bottleneck[81]. The mtDNA genetic bottleneck may occur as a result of either reduction of mtDNA copy number or amplification of a subset of mtDNA molecules. Regardless of the mechanism, the result of the bottleneck is to decrease the number of haplotypes within an egg. This phenomenon also increases genotypic variance between mature eggs, a feature that facilitates the rapid segregation of mtDNA variants between generations. During very early embryonic development, mtDNA replication is not active, and the mtDNA content per cell is diluted owing to an increase in cell number. The reduction in mtDNA content per cell, followed by resumption of mtDNA replication, has been proposed to result in a second bottleneck (part **c**) during early embryonic development[89]. The graph of mtDNA copy number illustrates trends during development; direct quantification is available for only selected stages.

of mtDNA content predicted by this model. Alternatively, selective replication and amplification of only a subpopulation of mtDNA molecules[81] may take place. The timing of mtDNA replication occurs independently of the cell cycle[82]. As a result, it is possible for a few mtDNA genomes in the developing oocyte to be preferentially replicated at the expense of others, leading to an apparent bottleneck. In this case, it is unclear whether this selective replication would be stochastic or biased towards particular mtDNA haplotypes.

Mitochondrial quality control in the female germ line. Genetic bottlenecks and genetic drift would result in oocytes with randomly skewed mtDNA genomes compared with the mtDNA population of primordial germ cells. However, two lines of research involving mice with pathogenic mtDNA mutations indicate the presence of a purifying selection process that monitors the quality and functionality of mtDNA genomes (FIG. 2). In the first set of studies, female mice that were heteroplasmic for a severe mtDNA mutation, such as a large deletion[83] or a frameshift mutation in the *ND6* gene[84], showed selective reduction of the pathogenic mitochondrial genomes during oogenesis. Offspring of such females had a lower mutation burden, and this tendency to reduce the mutational load enhanced with maternal age. By contrast, offspring born to mice heteroplasmic for non-pathogenic polymorphisms showed mutation burdens that were similar to the maternal burden. This suggests that oocytes containing severe mtDNA mutations are selectively eliminated from the germ line in an age-dependent manner. Further evidence for the existence of a mtDNA quality-control filter comes from the second set of studies, which analysed mice containing high levels of mtDNA mutations generated by a knock-in of an error-prone mtDNA polymerase. Female mice harbouring high levels of mtDNA mutations were mated with a wild-type male, and the range of mtDNA mutations in the offspring was compared with that in the mother. The offspring showed a strong suppression of mutations that change the coding sequence of protein-coding genes[85], which is a clear signature of genetic selection. Collectively, these studies point towards a process of purifying selection that helps to maintain the functionality of mtDNA in the female germ line and therefore in the offspring[86]. The presence of this filter probably explains why, in humans, most of the mutations causing familial encephalomyopathies are relatively mild, and most encephalomyopathies caused by large deletions are sporadic rather than familial[40]. mtDNA genomes with large deletions do arise *de novo* in somatic tissues with age[87], but these mutations are not passed through the germ line.

Little is known about the molecular mechanism of this quality-control filter. Even severe mtDNA mutations, such as deletions, do not cause OXPHOS defects until they accumulate beyond high thresholds[70–72]. In the studies described above using heteroplasmic mice, purifying selection is observed at mutation loads far lower than these thresholds[83,84], at least when the thresholds are assessed in commonly used cell models. Therefore, if the filter operates at the cellular level, it is surprisingly sensitive and probably involves a mechanism assessing a feature other than total cellular OXPHOS capacity. Because some mtDNA mutations increase the levels of reactive oxygen species (ROS), ROS sensing is one possible mechanism for this quality-control filter. Another possibility is that the filter is operating on the level of individual organelles, so that dysfunctional organelles containing pathogenic genomes might be segregated and eliminated, for example, by mitophagy.

A second genetic bottleneck during early embryogenesis. Between the zygote and the blastocyst stages[88], there is no mtDNA replication, which results in a mtDNA copy number reduction during early embryogenesis owing to dilution as cells rapidly divide. Later in development, mtDNA replication provides an increase in copy number, depending on tissue type. The results of experiments in rhesus monkeys suggest the existence of an additional genetic bottleneck during this early embryonic period before the resumption of mtDNA replication[89] (FIG. 2). When heteroplasmic oocytes are fertilized, the resulting eight-cell embryo shows a broad range of heteroplasmy in its individual blastomeres. However, later in development, fetuses arising from heteroplasmic oocytes show a tendency towards homoplasmy[89]. These observations suggest that somatic cells undergo a mtDNA bottleneck sometime after the formation of the blastocyst. By contrast, female germline cells in the fetus retain a broad range of heteroplasmy, suggesting that mtDNA inheritance in

Purifying selection
A mode of natural selection in which detrimental genetic variants are selected against.

somatic and germline lineages are differentially regulated during embryogenesis. Another experiment with heteroplasmic mice revealed that some somatic tissues can rapidly change the composition of their mtDNA[90]. In mice heteroplasmic for wild-type BALB/c and NZB mtDNA haplotypes, the blood and spleen invariably showed progressive accumulation of the BALB/c haplotype, whereas the kidney and liver showed accumulation of the NZB haplotype. The basis for this is unknown, but the two haplotypes do not result in differences in respiratory activity of the mitochondria[91]. These observations suggest that the genetic bottlenecks in somatic cells may include a considerable degree of complexity and tissue specificity.

Depletion of paternal mtDNA

A dramatic example of bias in mitochondrial segregation is the exclusively maternal inheritance of mitochondria in mammals. This has been recently further validated in humans by deep-sequencing techniques, which enabled the detection of even minor maternal genotypes but failed to detect any signatures of paternal mtDNA in the tissues of offspring[66].

Although uniparental inheritance of mtDNA is widespread in eukaryotes, the reason for its evolutionary conservation remains unclear. Most naturally occurring polymorphisms in mtDNA have negligible effects on mitochondrial function, and it is not obvious why it would be detrimental to have an equal mixture of two different, wild-type mtDNA genotypes (although, as mentioned above, humans normally do have low levels of heteroplasmy). A recent mouse study has provided striking evidence that substantial germline-inherited heteroplasmy can result in metabolic and behavioural aberrations[92]. Cytoplasts from the NZB mouse strain were fused with female embryonic stem cells from the 129S6 mouse strain to generate heteroplasmic stem cells, which were used to produce lines of heteroplasmic mice. When passed through the female germ line, a marked tendency towards the reduction of the NZB mtDNA genotype was observed, indicating that a bias in transmission between generations can occur even with two wild-type mtDNA genotypes. The NZB and 129S6 mtDNA genotypes are presumably equivalent in function because mice homoplasmic for either show indistinguishable levels of activity, fertility and behaviour. However, when NZB–129S6 heteroplasmic animals were compared with their homoplasmic NZB or 129S6 littermates, several metabolic and behavioural changes were noted, including reduced physical activity and lower food intake. In addition, they showed aberrations in tests that measure anxiety and spatial learning. This mouse model therefore provides evidence that heteroplasmy can have metabolic and behavioural effects, and indicates that even apparently equivalent wild-type mitochondrial genomes can be distinguished during germline inheritance[92]. Uniparental inheritance of mtDNA may therefore be advantageous by promoting homoplasmy and avoiding such phenotypes in the offspring. The molecular mechanisms underlying these heteroplasmic defects are unknown but may be related to subtle differences in bioenergetic properties or ROS generation between the two mtDNA haplotypes.

An early hypothesis suggested that uniparental inheritance of mitochondria may be a passive consequence of the asymmetry in male and female gamete size; for example, in mammals, the male gamete is much smaller and contains considerably fewer mitochondria than the oocyte. However, some organisms, such as the unicellular green alga *Chlamydomonas reinhardtii*, have equal-sized gametes but nevertheless show uniparental inheritance of mtDNA[93]. As discussed below, recent studies indicate that active mechanisms that function pre- and post-fertilization have evolved to ensure uniparental inheritance of mtDNA. There are indications that these mechanisms can occasionally go awry or be bypassed. For example, there is a single clinical case reported of a patient with exercise intolerance who inherited mtDNA haplotypes from both his mother and father, with the paternal haplotype accumulating to high levels in skeletal muscles[94]. The patient's myopathy resulted from an apparent *de novo* mutation that arose in the paternal mtDNA.

Pre-fertilization depletion of paternal mtDNA. In *D. melanogaster*, mtDNA is eliminated from spermatozoa as they develop from 'onion stage' spermatids to fully elongated spermatids that are subsequently individualized (FIG. 3a). Spermatid maturation takes place within a syncytium; sperm individualization is a process that is necessary for generating individual gametes, whereby each spermatid is packaged into a separate plasma membrane, separating it from the syncytium. During the final stage of spermatid elongation, the mtDNA is progressively removed from the nuclear end towards the tail[95]. By the time spermatids are fully elongated, few mtDNA nucleoids persist. As a result, mature sperm in *D. melanogaster* normally have little, if any, mtDNA by the time of fertilization.

This degradation process is dependent on Endonuclease G (EndoG), one of five nucleases with mitochondrial targeting signals[95]. In EndoG mutants, mtDNA nucleoids aberrantly persist in the tail regions of fully elongated and individualized spermatids. Nevertheless, eggs fertilized by EndoG-mutant males do not contain detectable paternal mtDNA. Analysis of EndoG mutants reveals a redundant process in later spermatid development that further eliminates mtDNA from the sperm. When sperm undergo individualization in EndoG-mutant flies, the residual mtDNA in the spermatid tail is transported, along with other cellular components, into a waste compartment that is culled from the spermatid (FIG. 3a). It will be interesting to determine whether similar pre-fertilization mechanisms occur in mammals. Indeed, in mice there is a tenfold decrease in mtDNA levels as sperm mature[96], but the molecular basis of this is unknown.

Post-fertilization depletion of paternal mtDNA. In bovine and primate zygotes, the paternal mitochondria can be selectively stained using ubiquitin antibodies, and ubiquitin has been proposed to be a mark that targets the paternal organelles for proteasomal destruction[97]. According to this hypothesis, mitochondrial ubiquitylation is carried out in the sperm, and no active mechanism in the egg would be needed to mark the paternal mitochondria for post-fertilization destruction[97].

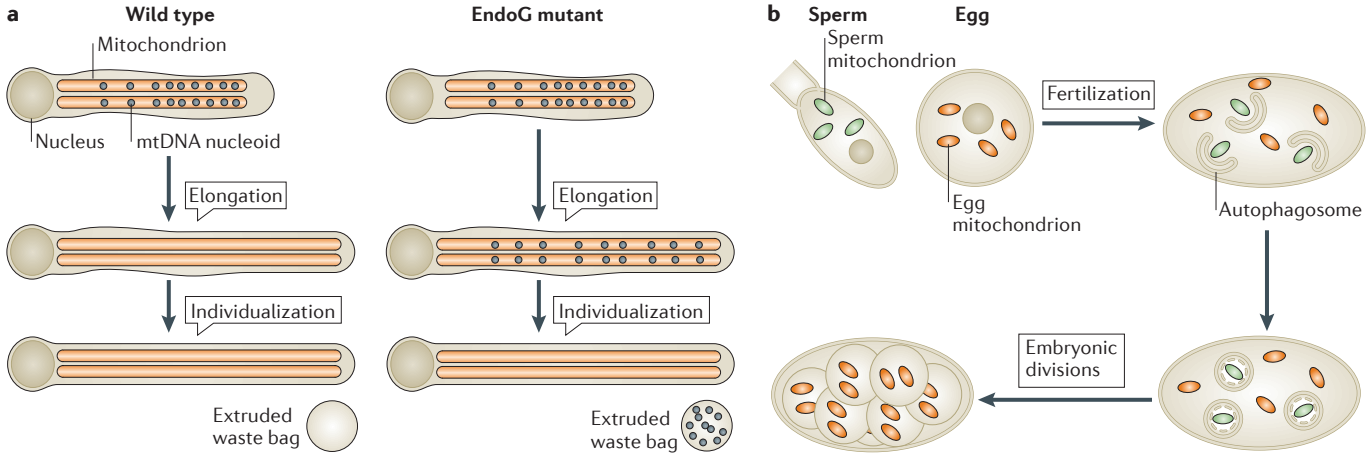


Figure 3 | **Mechanisms ensuring the elimination of paternal mtDNA.** **a** | Pre-fertilization mechanisms in flies. In *Drosophila melanogaster*, two mechanisms remove mitochondrial DNA (mtDNA) from sperm. Left: mtDNA is normally degraded during sperm elongation by mitochondrial Endonuclease G (EndoG). Right: In EndoG-mutant flies, the mtDNA persists beyond the elongation stage but is ultimately removed by a second mechanism (extrusion) during the individualization stage, in which mtDNA and cellular debris are sequestered into a waste compartment that is extruded from the sperm body. **b** | Post-fertilization mechanism in the nematode *Caenorhabditis elegans*. Shortly after fertilization, paternal mitochondria colocalize with autophagy markers (not shown) and are eliminated by mitophagy by the 16–64 cell stages. Worms deficient for autophagy show persistence of paternal mitochondria long beyond this stage.

Genetic studies in *Caenorhabditis elegans* clearly indicate a role for mitophagy in the removal of paternal mitochondria after fertilization[98–100]. Shortly after fertilization, autophagosomes are recruited to paternal mitochondria, and consequently they disappear by the 16-cell or the 64-cell stages[98,99] (FIG. 3b). Mutants in the autophagy pathway show persistence of paternal mitochondria well beyond these stages[98,99]. Similar results were obtained in embryos treated with inhibitors of lysosomal function[100]. In contrast to those in mammals, the sperm mitochondria in nematode embryos are not polyubiquitylated[98,99]. Nevertheless, nematodes with mutations in proteasome ubiquitin receptors are impaired in the degradation of their paternal mitochondria[100].

Fertilized mouse oocytes show colocalization of the autophagic markers LC3, GABARAP and p62 with the paternal mitochondria[98], suggesting that autophagy is a conserved mechanism for paternal mtDNA depletion in mammals as well. However, this view has been challenged by the recent observation that the association of LC3 and p62 with paternal mitochondria is transient and occurs long before the loss of mitochondria[101]. As a result, it has been suggested that elimination of mtDNA in mammalian sperm is a passive process and is largely accomplished before fertilization by reduction of mtDNA in sperm[101]. In addition, mitochondrial partitioning in the dividing blastomeres is uneven, and this may remove the remaining paternal mtDNA from most tissues of the embryo[101]. More work is needed in order to resolve the mechanism of mammalian uniparental mtDNA inheritance.

Concluding remarks

In mammalian cells, the partitioning of mitochondria to daughter cells during cell division seems to involve passive mechanisms. Mitochondria undergo fragmentation during mitosis and are well distributed throughout the cell soma owing to their interactions with the ER and cytoskeleton. Thus, cytokinesis can result in the partitioning of roughly equal amounts of mitochondria to daughter cells. The role of active transport processes, such as the Milton–Miro–KIF5B complex, in mitochondrial partitioning during cell division has not been examined. Active partitioning mechanisms have been delineated in budding yeast; it seems likely that proliferating mammalian cells would use analogous mechanisms, but so far there are no data to support this. Because cells do not survive without mitochondria, the availability of temperature-sensitive mutants in *Saccharomyces cerevisiae* has been instrumental in identifying mitochondrial transport mechanisms to the bud. To make further progress in our understanding of mammalian mitochondrial partitioning, new assays and model systems will need to be developed to analyse mitochondrial distribution, heterogeneity and segregation in mammalian cells, as well as to study asymmetric cell division.

During maternal transmission of mitochondria, the mtDNA genetic bottleneck and purifying selection during oogenesis have major influences on the physiology of offspring and potentially on the overall evolution of species. The molecular mechanisms underlying these complex phenomena are poorly understood, and it is unknown whether the mechanisms regulating mitochondrial partitioning in cell division are relevant to understanding mitochondrial transmission between generations. A future challenge will be to synthesize these parallel lines of research. Mechanisms of mitochondrial quality control are another area for future exploration. Several pathways of mitophagy have been identified so far, and it will be important to understand the extent to which these pathways contribute to maintenance of mitochondrial function in health and disease.

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Competing interests statement
The authors declare no competing interests.