REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

MECHANISMS OF DISEASE

Mitochondrial Dynamics — Mitochondrial Fission and Fusion in Human Diseases

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Mitochondria are mobile organelles that exist in dynamic networks. They continuously join by the process of fusion and divide by the process of fission. Mitochondria are derived from eubacterial endosymbionts that are capable of aerobic respiration; this finding was proposed independently by Merezhkovsky in 1905 and by Margulis in 1967.[1] First described as "bioblasts" by Altmann in 1890, it was Benda's 1898 observation of their heterogeneous morphologic features, sometimes ball-shaped and other times linear, that inspired the name mitochondrion, from the Greek words mitos (meaning thread) and chondrion (meaning granule).[2] Lewis and Lewis's 1914 observations established the field of mitochondrial dynamics. They noted, "Any one type of mitochondria such as a granule, rod or thread may at times change into any other type or may fuse with another mitochondrium [sic], or it may divide into one or several mitochondria."[3] The once secret, dynamic lives of mitochondria are revealed by confocal, live-cell imaging with the use of potentiometric dyes or mitochondria-targeted fluorescent proteins (see Fig. 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

Most mitochondrial proteins, including all those involved in fission and fusion, are nuclear-encoded.[4] Mutations in 228 nuclear and 13 mitochondrial genes cause rare monogenic syndromes in which mitochondrial dysfunction is unequivocally central to the pathogenesis of the disease. Examples of these syndromes include the MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes caused by mutation of mitochondrial transfer RNAs), and Leigh's disease (caused by mutations in genes related to oxidative phosphorylation).[4] In contrast, disorders of mitochondrial structure are just emerging as mechanisms of disease. Although some disorders of mitochondrial dynamics result from monogenic mutation, most reflect changes in the function or activity of fission and fusion mediators triggered by changes in the cellular milieu. There is an emerging recognition that disordered mitochondrial dynamics contribute to the pathogenesis of complex diseases that are not classically considered to involve mitochondria; these diseases include cancer, cardiovascular disease, and neurodegenerative diseases. Recent identification of the molecular mediators of mitochondrial dynamics (Table 1) and recognition of their post-translational regulation by an extensive network of kinases, phosphatases, and ubiquitination mediators offer a new understanding of cell biology and novel therapeutic targets. Fission and fusion fine-tune fundamental cellular processes such as calcium homeostasis and the generation of ATP and reactive oxygen species and consequently have important roles in cell-cycle progression, apoptosis, mitophagy, and oxygen sensing.

Although ATP generation is the primary function of the mitochondria (Fig. 1), much of the impact of mitochondrial dynamics relates to effects of structure on

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the nonbioenergetic capabilities of the organelle. Five noncanonical mitochondrial capabilities are centrally involved in the form–function dynamic of the mitochondria (Fig. 2). These relationships are bidirectional, meaning they both alter and are altered by mitochondrial dynamics. First, mitochondria are linked to the endoplasmic reticulum at specialized regions of membrane adherence called mitochondria-associated membranes, which facilitate calcium flux into the mitochondria. Mitochondria-associated endoplasmic reticulum membranes allow localized increases in calcium levels to propagate throughout a cell in wavelike patterns.[49] This endoplasmic reticulum–mitochondria connection has implications for calcium homeostasis and metabolism. Close coupling of these organelles increases mitochondrial calcium levels, which may initiate apoptosis[50] or, at physiologic levels, enhance oxidative metabolism by activating pyruvate dehydrogenase.[51] Mitochondria–endoplasmic reticulum connectivity is regulated by mitofusin-2 and can create microdomains that facilitate fission[9,27] (Fig. 2B, inset).

Second, mitochondrial numbers are regulated by mitochondrial biogenesis to meet the energy demands of the cell and compensate for cell damage. This process is mediated by peroxisome-proliferator–activated receptor γ coactivator 1α (PGC-1α), which is relevant to mitochondrial dynamics since it is a transcriptional coactivator of the fusion mediator mitofusin-2 (Table 1).[12,29]

Third, mitochondria have a quality-control program called mitophagy. Mitophagy maintains cellular health by selectively enclosing damaged, depolarized mitochondria in autophagic vacuoles for elimination by lysosomes (Fig. 3A).[52,53] Fission isolates depolarized mitochondria while coordinated down-regulation of fusion mediators prevents network reintegration, thereby facilitating mitophagy.

Fourth, mitochondria actively traverse the cytosol on dynein and kinesin tracks.[54] It is uncertain whether mobility and mitochondrial dynamics have an obligatory relationship; however, dynein, a molecular motor for mitochondrial transport, also regulates fission.[55]

Fifth, mitochondria are oxygen sensors in cells within the homeostatic oxygen-sensing system, such as pulmonary arterial and ductus arteriosus smooth-muscle cells.[56] These specialized mitochondria vary production of diffusible reactive oxygen species by the electron transport chain in proportion to cellular oxygen levels, permitting redox regulation of ion channels, enzymes, and transcription factors.[56] Mitochondrial dynamics are an early step in this redox signaling mechanism.[10,23]

Fission creates smaller, more discrete mitochondria, which, depending on the context, are more capable of generating reactive oxygen species, facilitating mitophagy, or accelerating cell proliferation. Fusion results in a more interconnected mitochondrial network that enhances communication with the endoplasmic reticulum. Fusion also allows diffusion of matrix content among mitochondria (Fig. 2A), diluting the accumulated mitochondrial DNA mutations[57] and oxidized proteins. Both fission and fusion are mediated by a small number of highly conserved, guanosine triphosphatases (GTPases)[58,59] (Fig. 2 and Table 1). Fission is mediated by dynamin-related protein 1 (DRP1),[60,61] a cytosolic protein that on activation translocates to the outer mitochondrial membrane. Here, DRP1 multimerizes, creating a ringlike structure that constricts and divides the organelle[21,22] (Fig. 2B). Video 1, available at NEJM.org, shows the dynamic nature of fission and fusion when a photoactivated green fluorescent protein–labeled mitochondrion divides (fission) and then fuses with a red fluorescent protein–labeled mitochondrion to create a fused yellow mitochondrion. DRP1 is actively targeted to the outer mitochondrial membrane by non-GTPase receptor proteins such as mitochondrial fission protein 1 (Fis1),[22] mitochondrial fission factor (MFF),[25] and mitochondrial elongation factor 1[26] (Fig. 2B and Table 1). Assembly of the fission apparatus is assisted by the endoplasmic reticulum, which contacts the mitochondria, creating a microdomain for assembly of DRP1, MFF, and proapoptotic proteins[62] (Fig. 2B and 3A).

DRP1 activity is rapidly regulated by the opposing effects of phosphorylation at two key serines. Phosphorylation of serine 616 increases DRP1 activity, whereas phosphorylation of serine 637 decreases it. Each serine is targeted by different kinases and phosphatases, thereby linking mitochondrial fission to crucial cellular processes (Fig. 2B, inset; and Table 1). For example, phosphorylation of serine 616 by the mitosis initiator, cyclin B1–cyclin-dependent kinase (cyclin B1–CDK1), activates DRP1 coordinating fission to cell division[23,32] (Fig. 3B), whereas phosphorylation by calcium-calmodulin–dependent kinase

A video showing mitochondrial dynamics is available at NEJM.org

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(CamK) links fission to intracellular calcium.[34] Phosphorylation of serine 637 by protein kinase A inactivates DRP1[63]; conversely, dephosphorylation of serine 637 by the calcium-sensitive protein phosphatase, calcineurin, activates DRP1 (Fig. 2B and Table 1). The ratio of serine 616 to serine 637 phosphorylation determines DRP1 activity and reflects the aggregate effects of many kinases and phosphatases (Fig. 2). DRP1 activity is also post-translationally regulated by the ubiquitin ligase membrane–associated RING-CH protein 5 (MARCH5)[38] and by small ubiquitin-like modifier type 1 (SUMO1)[37] (Table 1 and Fig. 2B). Our understanding of the role of DRP1 and fission in disease states has been advanced by the availability of mitochondrial division inhibitor 1 (mdivi-1), a small molecule that inhibits the GTPase activity of DRP1, prevents multimerization, and inhibits fission.[64]

Fusion is mediated by mitofusin-1 and mitofusin-2 isoforms in the outer mitochondrial membrane and by optic atrophy 1 (OPA1) protein in

Table 1. Fission and Fusion Mediators.*

Mediator	Mediator Function	Human Disease Related to Mutation	Human Disease Involving Acquired Abnormalities of Mitochondrial Dynamics
Fusion mediators			
Mitofusin-1[5,6]	GTPase in outer mitochondrial membrane that tethers adjacent mitochondria	Unknown	
Mitofusin-2[7]	GTPase in outer mitochondrial membrane that tethers adjacent mitochondria	Charcot–Marie–Tooth disease type 2A[8]	Pulmonary arterial hypertension,[9,10] lung cancer,[11] arterial restenosis[7,12]
Optic atrophy 1[13]	GTPase in inner mitochondrial membrane that mediates fusion of inner mitochondrial membrane	Optic atrophy[14]	Hypertension[15]
Fission mediator: DRP1[21,22]	Cytosolic GTPase that translocates to the outer mitochondrial membrane when activated	Congenital microcephaly, lactic acidosis, sudden death[16]	Parkinson's disease,[17-19] Huntington's disease,[20] patent ductus arteriosus,[10] pulmonary arterial hypertension,[12,23] lung cancer[11,24]
DRP1 targeting proteins			
Mitochondrial fission 1 protein[22]	DRP1-targeting protein in the outer mitochondrial membrane that recruits DRP1 from the cytosol to the mitochondria	Unknown	Unknown
Mitochondrial fission factor[25]	DRP1-targeting protein in the outer mitochondrial membrane that recruits DRP1 during mitochondrial fission	Unknown	Unknown
Mitochondrial elongation factor 1[26]	DRP1- targeting protein in the outer mitochondrial membrane that recruits DRP1 to mitochondria but inhibits its function, promoting fusion	Unknown	Unknown
Fusogenic and fissogenic lipids			
Phosphatidic acid[27]	Generated by mitochondrial phospholipase D; promotes assembly of fusogenic mediators	Unknown	Unknown
Diacylglycerol[28]	Lipin-1, a protease that hydrolyzes phosphatidic acid, generates diacylglycerol, which promotes fission	Unknown	Unknown
Transcription factors			
PGC-1α[12,29]	Mediator of mitochondrial biogenesis and transcriptional coactivator of mitofusin-2	Unknown	Obesity and diabetes,[30] pulmonary arterial hypertension[12]
Hypoxia-inducible factor 1α[23,31]	Hypoxic transcription factor that also promotes DRP1 activation and fission (mechanism uncertain)	Unknown	Pulmonary arterial hypertension,[9,10] cancer[11,24]

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Table 1. (Continued.)

Mediator	Mediator Function	Human Disease Related to Mutation	Human Disease Involving Acquired Abnormalities of Mitochondrial Dynamics
Post-translational regulators of DRP1			
Cyclin B–cyclin-dependent kinase 1[23,32]	Serine–threonine kinase that initiates mitosis and also activates DRP1 by phosphorylation of DRP1 serine 616	Unknown	Pulmonary arterial hypertension,[23] patent ductus arteriosus[10]
Aurora A kinase[33]	Serine–threonine kinase activated in the G2–M phase of the cell cycle, regulating mitotic entry, chromosomal segregation, and DRP1 activation		Cancer[33]
Calcium-calmodulin–dependent kinase[34]	Activates DRP1 (mechanism uncertain)	Unknown	Patent ductus arteriosus[10]
Calcineurin[35]	Calcium-dependent, serine–threonine protein phosphatase that activates DRP1 by dephosphorylating DRP1 serine 637	Unknown	Ischemia–reperfusion injury[36]
Protein kinase A[35]	Causes cyclic AMP–dependent phosphorylation of DRP1 at serine 637, which inhibits fission	Unknown	Unknown
SENP5[37]	Nucleolar small ubiquitin-like modifier protease that moves to the mitochondria during mitosis and desumoylates DRP1, which leads to the activation of DRP1	Unknown	Unknown
MARCH5[38]	Ubiquitin ligase that modulates mitofusins and DRP1	Unknown	Unknown
Mitochondrial kinases relevant to mitophagy			
PTEN-induced kinase 1[39-41]	Serine–threonine kinase that regulates mitochondrial quality control and targeting of parkin	Autosomal recessive juvenile parkinsonism[42]	Unknown
Parkin[16,17,43,44]	Ubiquitin E3 ligase that regulates mitochondrial quality control[45-48]	Autosomal recessive juvenile parkinsonism[45-48]	Unknown

* DRP1 denotes dynamin-related protein 1, MARCH5 membrane-associated RING-CH protein 5, and PTEN phosphatase and tensin homologue.

the inner mitochondrial membrane[13] (Table 1 and Fig. 1A). Mitofusins are targeted to the mitochondria by sequences in their transmembrane and C-terminal domains.[65] With cytosolic amino and carboxyl termini, mitofusins initiate fusion by creating homodimeric or heterodimeric, antiparallel, coiled-coil linkages between adjacent mitochondria[5] (Fig. 2A, inset). Mitofusin-2 is also located in the endoplasmic reticulum, where it alters morphologic features and promotes endoplasmic reticulum–mitochondrial tethering,[65] thereby enhancing mitochondrial calcium uptake.[9] OPA1 has eight splice variants, each with differential fusion activity and mitochondrial protease susceptibility.[6] The fusion mediators also regulate mitochondrial metabolism, and when they are down-regulated or dysfunctional, there is generally a reduction in mitochondrial oxidative capacity.[6] Fusion and fission are guided by lipids generated by mitochondrial phospholipase D, notably phosphatidic acid.[27] The small, negatively charged lipid head group of phosphatidic acid causes negative curvature of lipid bilayers and recruits adaptor proteins, promoting fusion.[27] However, phosphatidic acid can be hydrolyzed by lipin-1, creating diacylglycerol, which promotes fission.[28]

DISEASES INVOLVING MITOCHONDRIAL DYNAMICS

Disorders of mitochondrial dynamics are implicated in neurodegenerative, neoplastic, endocrine, and cardiovascular diseases. The therapeutic implications of the mitochondrial dynamics pathway for these diseases are reviewed in Figure 4.

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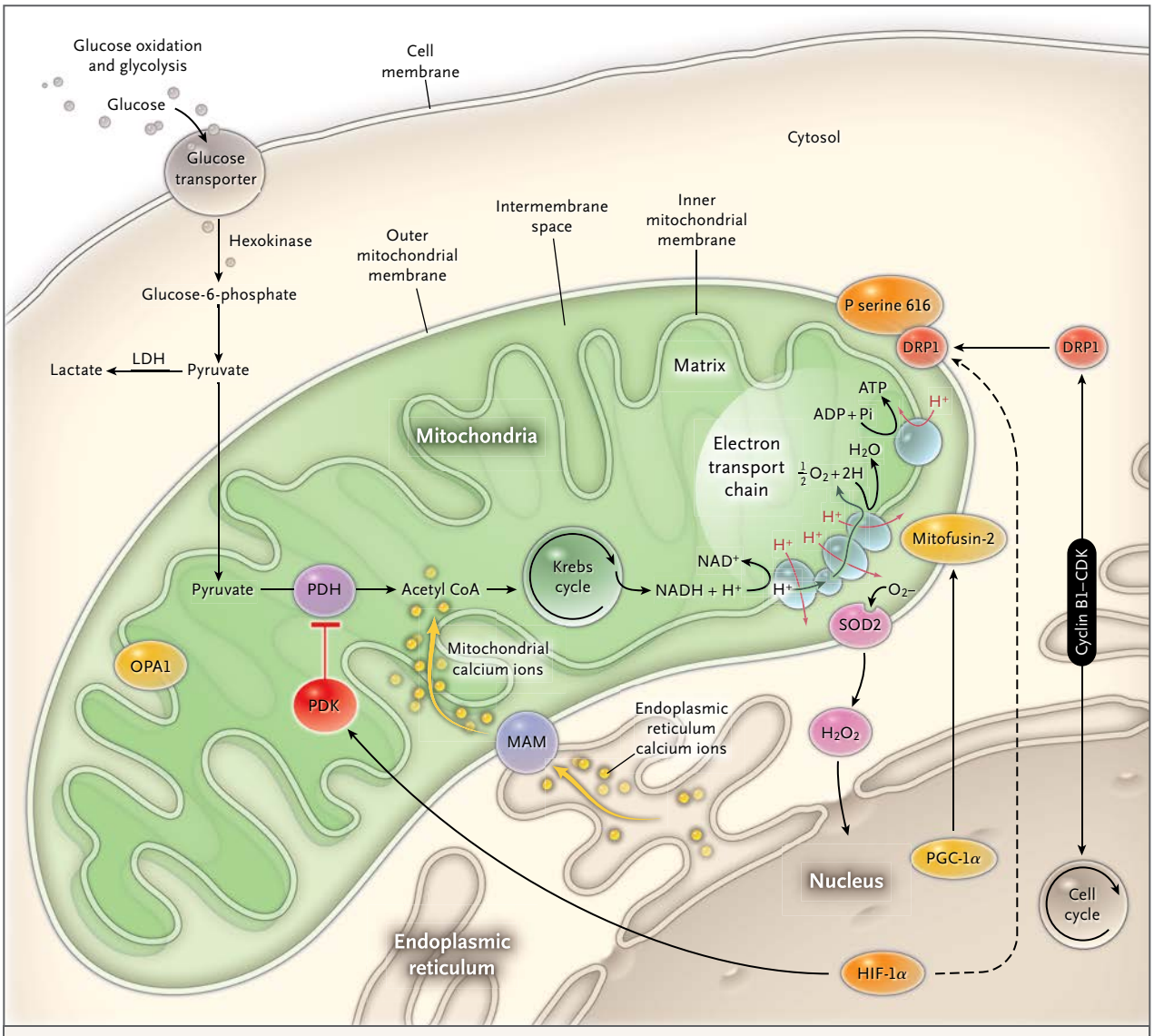

Figure 1. Mitochondrial Metabolism and Dynamics.

The permeable outer mitochondrial membrane and impermeable inner membrane are separated by an intermembrane space into which hydrogen ions (H+) are pumped during electron transport. Within the inner mitochondrial membrane is a matrix containing mitochondrial DNA, proteins, and ribosomes. Within the cristae reside the four electron transport chain megacomplexes and the ATP-synthase, where ADP and inorganic phosphate (P_i) are joined to make ATP. Pyruvate generated in the cytosol by glycolysis either enters the mitochondria and fuels oxidative metabolism or remains in the cytosol and is converted to lactate by lactate dehydrogenase (LDH) if mitochondrial metabolism is inhibited. Once inside the mitochondria, pyruvate is converted into acetyl coenzyme A (CoA) by pyruvate dehydrogenase (PDH), the major regulator of oxidative metabolism. PDH can be inhibited by pyruvate dehydrogenase kinase (PDK) or activated by increases in mitochondrial calcium. Acetyl CoA fuels the Krebs cycle and generates electron donors (NADH and flavin adenine dinucleotide), which pass electrons down the electron transport chain to reduce molecular oxygen. This electron flux generates the chemiosmotic hydrogen ion gradient that powers ATP synthesis. In addition, electron flux generates reactive oxygen species such as superoxide anion (O_2–), which are converted to the diffusible redox signaling molecule hydrogen peroxide (H_2O_2) by superoxide dismutase 2 (SOD2). Mediators of fusion (mitofusin-1 and mitofusin-2) and the enzyme that generates fusogenic lipids, phospholipase D, reside in the outer mitochondrial membrane, whereas optic atrophy 1 (OPA1) is located in the inner membrane. The fission mediator DRP1 is located in the cytosol, but when it is activated, as it is after phosphorylation (P) at serine 616, it translocates to the outer mitochondrial membrane. The cell cycle and mitochondrial fission are linked by cyclin B1–cyclin-dependent kinase (CDK), ensuring coordination of nuclear and mitochondrial division. Peroxisome-proliferator–activated receptor γ coactivator 1α (PGC-1α) and hypoxia-inducible factor 1α (HIF-1α) also provide transcriptional control of fission and fusion. Calcium moves from the endoplasmic reticulum to mitochondria through membrane-adherence zones called mitochondria-associated endoplasmic reticulum membranes (MAMs). Mitochondrial calcium levels regulate PDH activity and apoptosis. The mitochondria and endoplasmic reticulum also interact to create microenvironments that direct fission.

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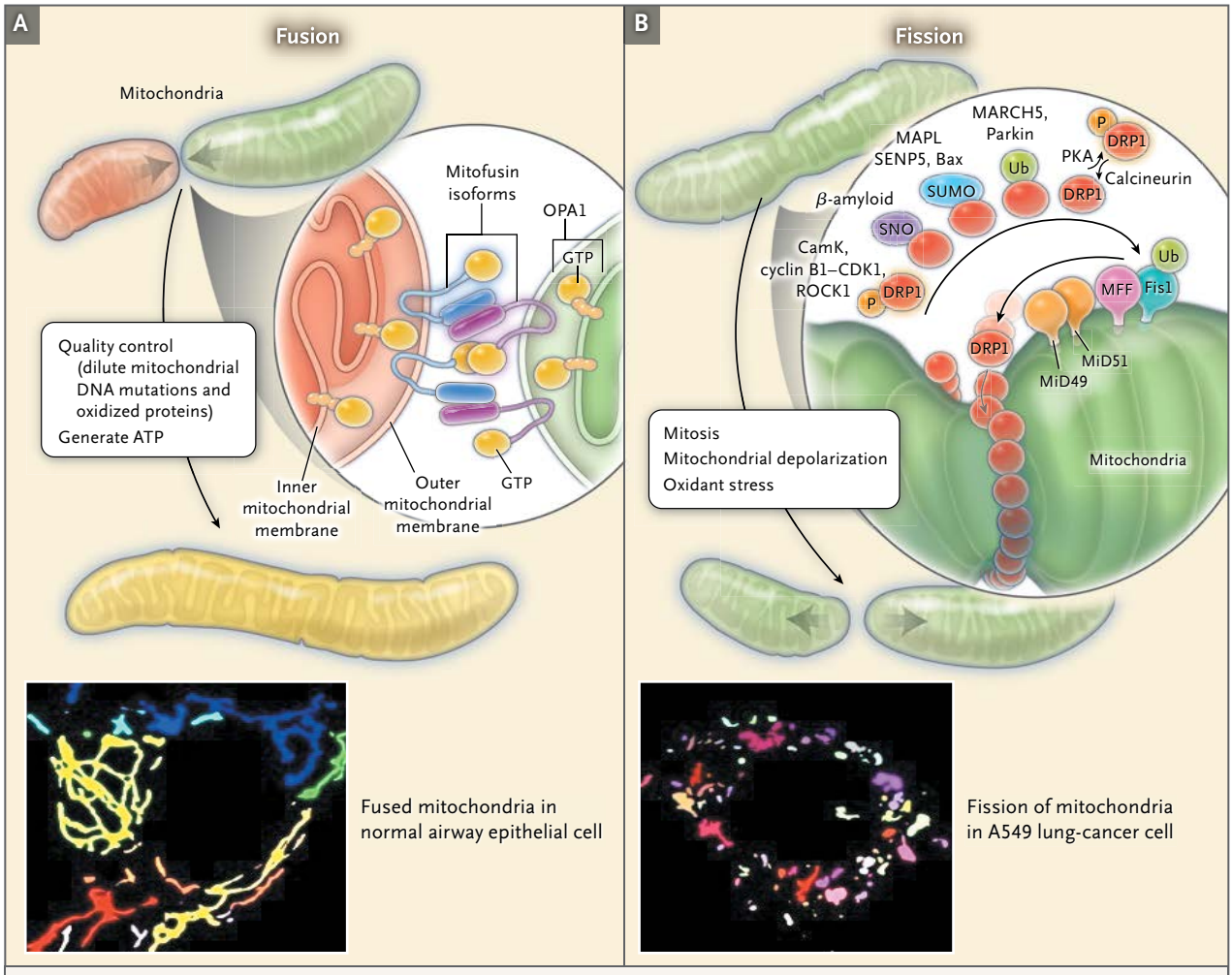

Fused mitochondria in normal airway epithelial cell

Fission of mitochondria in A549 lung-cancer cell

Figure 2. Mitochondrial Fusion and Fission.

Panel A shows how mitochondria join through the process of fusion, which is mediated by the coordinated activities of mitofusins in the outer mitochondrial membrane and OPA1 in the inner mitochondrial membrane. The mitochondrial matrix contains red or green fluorescent proteins. When mitochondria fuse, their matrix materials intermix, creating elongated organelles that appear yellow. The inset shows how the two mitofusin isoforms form homodimers or heterodimers that tether adjacent mitochondria, promoting fusion by a hook-and-loop mechanism that is similar to a Velcro fastener. Panel B shows how fission occurs when DRP1 is activated and moves from the cytosol to the outer mitochondrial membrane. Assembly of the fission apparatus is guided by targeting molecules in the outer mitochondrial membrane (mitochondrial fission factor [MFF] and mitochondrial fission 1 protein [Fis1]) that are aggregated in a microenvironment shaped by contact with the endoplasmic reticulum. Activation usually reflects post-translational modification by a kinase, phosphatase, or ubiquitin ligase, or by alteration of sumoylation. For example, calcium-calmodulin–dependent kinase (CamK) and cyclin B1–CDK1 phosphorylate and activate DRP1. Nitric oxide may activate DRP1 by means of nitrosylation. The nucleolar small ubiquitin-like modifier (SUMO) protease sentrin-specific peptidase 5 (SENP5) can activate DRP1 by means of desumoylation. Membrane-associated RING-CH protein 5 (MARCH5) and parkin are ubiquitin ligases that can regulate DRP1 activation and degradation. Mitochondrial dynamics protein 49 kD (M:D49) and mitochondrial dynamics protein 51 kD (M:D51), which are located in the mitochondrial outer membranes, form division-inducing rings around mitochondria. The formation of rings that cause fission is a property shared by DRP1. Protein kinase A (PKA) phosphorylates DRP1 at serine 637, which inactivates the enzyme; conversely, calcineurin reverse-dephosphorylates this serine, which activates DRP1. The insets show confocal micrographs in which color coding identifies discrete mitochondria. The normal airway epithelial cell has fused mitochondria, and its cancerous counterpart, an A549 lung-cancer cell, shows evidence of mitochondrial fission. Bax denotes Bcl-2–like protein 4, ROCK1 Rho-associated, coiled-coil–containing protein kinase 1, MAPL mitochondrial-anchored protein ligase, SNO S-nitrosylated Drp1, and Ub ubiquitin. Adapted from Rehman et al.[11]

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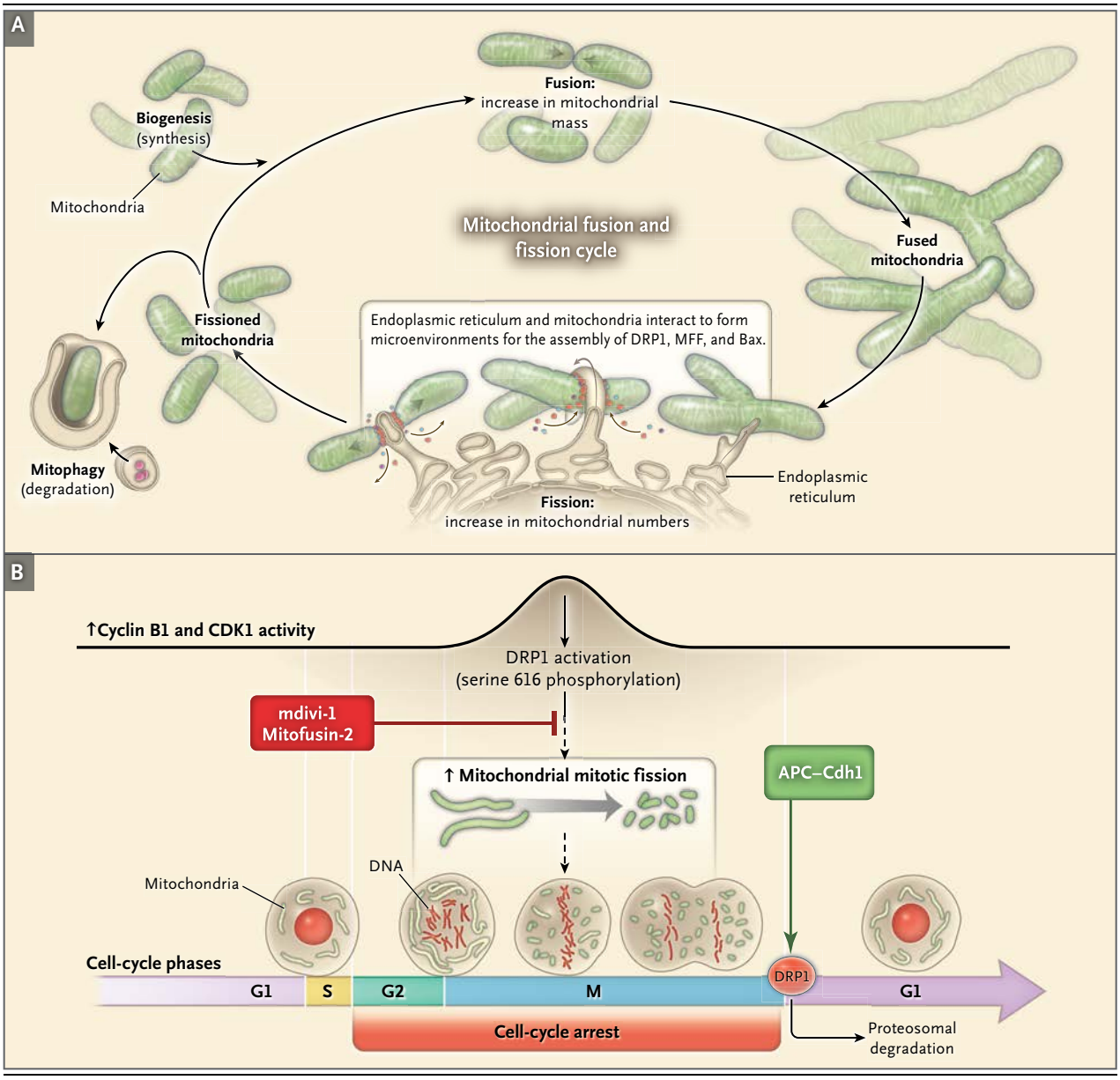

PROLIFERATIVE, APOPTOSIS-RESISTANT DISEASES

Cancer

Excessive proliferation and resistance to apoptosis are hallmarks of cancer cells (Fig. 4A).[69] These hallmarks reflect, in part, acquired abnormalities of mitochondrial function, notably a shift from oxidative metabolism to aerobic glycolysis (the Warburg effect).[70] This metabolic phenotype primarily reflects activation of mitochondrial pyruvate dehydrogenase kinase, which inhibits pyruvate dehydrogenase and suppresses oxidative metabolism, rendering the cell apoptosis-resistant (Fig. 1). These functional mitochondrial abnormalities offer potential therapeutic targets. Restoring pyruvate dehydrogenase activity and oxidative metabolism inhibits proliferation, induces apoptosis, and shrinks human tumors in xenotransplantation models[41] and in patients.[71] Structural mitochondrial abnormalities also contribute to the imbalance between proliferation and apoptosis in cancer.[11] Impaired fusion and enhanced fission fragment the mitochondrial network in lung adenocarcinoma in humans[11] (Fig. 4A).

The role of mitochondrial dynamics in cancer

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Figure 3 (facing page). Noncanonical Functions of Mitochondria Related to Mitochondrial Dynamics.
Panel A shows mitochondrial function in nonmitotic cells. Mitochondria are actively regulated to prevent cell damage and death. Several responses can be deployed, sequentially or in tandem, to protect cells. For instance, PGC-1α–mediated mitochondrial biogenesis increases mitochondrial numbers and replaces damaged mitochondria. In addition, mitofusin-mediated fusion dilutes damage that accumulates in the mitochondria, including mitochondrial DNA mutations and oxidized proteins. When mitochondrial damage exceeds a critical level, DRP1-mediated asymmetric fission supports mitochondrial quality control. DRP1 divides a mitochondrion into a larger, polarized healthy portion (which can reintegrate with the network) and a smaller depolarized, abnormal portion. These depolarized mitochondrial segments have lower levels of fusion mediators, such as OPA1, preventing reincorporation into the network. This isolates the diseased mitochondria, allowing them to be packaged into autophagic vacuoles that are transported to lysosomes for elimination by mitophagy. These responses are beneficial only when they operate at a physiologic level. Excessive fission causes cell death, whereas excessive mitophagy causes autophagic stress; both promote cell damage and death. Mitochondria–endoplasmic reticulum interactions occur at sites of imminent fission, creating a microdomain for assembly of the fission apparatus.[27] Panel B shows a dividing cell. Mitosis is coordinated with mitochondrial division to ensure the equitable distribution of mitochondria to daughter cells; this process is called mitotic fission. Although several kinases, including aurora kinases, are involved, the role of cyclin B1–CDK1 has been studied most extensively. Cyclin B1–CDK1 simultaneously promotes nuclear division (mitosis) and phosphorylates and activates DRP1 at serine 616, causing fission. Ubiquitination and sumoylation also regulate mitotic fission.[37] During mitosis, SENP5, a nucleolar SUMO protease, moves to the mitochondria, where it desumoylates and activates DRP1.[37] After mitosis, ubiquitination by the anaphase-promoting complex (cyclosome) and its coactivator cadherin 1 (APC–Cdh1) marks DRP1 for proteosomal degradation, allowing G1-phase reassembly of mitochondrial networks.[44] If mitochondria are forced to remain fused (through inhibition of DRP1-using small interfering RNA [siRNA] or mitochondrial division inhibitor 1 [mdivi-1] or through augmentation of mitofusin-2), the cell cycle is arrested in the G2–M phase and the cells tend to be eliminated by apoptosis. Thus, mitotic fission is a cell-cycle checkpoint and inhibition of mitotic fission impairs cell-cycle progression.

relates to the requirement for mitochondrial division during mitosis. This coordination between mitochondrial division and mitosis (so-called mitotic fission) ensures equitable distribution of mitochondria to daughter cells[32,43] (Fig. 3B). The molecular basis for the coordination of mitochondrial and nuclear division is emerging. At the transition from the G1 phase to the S phase, mitochondria fuse and increase ATP production.[43] DRP1 inhibition induces mitochondrial hyperfusion and triggers DNA replication and cyclin E accumulation.[43] The coordination of fission and mitosis is substantially regulated by cyclin B1–CDK1, which simultaneously initiates mitosis and activates DRP1 by phosphorylating serine 616[11] (Fig. 2B). Another mitotic kinase, aurora A, phosphorylates the Raslike GTPase (RalA), leading to mitotic, mitochondrial accumulation of RalA and its effector, ralA binding protein 1 (RalBP1). RalBP1 serves as a scaffold for recruiting DRP1 and cyclin-CDK to mitochondria and inducing fission.[33]

Increased fission in lung-cancer cells and tumors from patients who have not received treatment reflects post-translational DRP1 activation, manifested by an increased ratio of serine 616 to serine 637 phosphorylation.[11] In patients with lung cancer, increased DRP1 expression predicts a likelihood of recurrence that is increased by a factor of 3.4, as well as a greater likelihood of cisplatin resistance.[24] Impaired fusion, resulting from down-regulation of mitofusin-2, also contributes to network fragmentation.[11] Mitochondrial fragmentation contributes to the cancer phenotype in several ways. Fragmentation may accelerate mitotic fission[11] and also interrupts intramitochondrial calcium waves, preventing calcium-mediated apoptosis.[50] Inhibition of DRP1 or augmentation of mitofusin-2 decreases proliferation and increases apoptosis in cancer cells and leads to regression of human lung tumors in a xenotransplantation model[11] (Fig. 4A).

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (Fig. 4B) is an obstructive pulmonary vasculopathy. Although vasoconstriction, inflammation, and thrombosis contribute to the pathogenesis, an "oncologic view" is emerging, which holds that excessive proliferation and impaired apoptosis mediate disease progression.[17] Many factors contribute to the neoplastic phenotype of pulmonary arterial hypertension.[18,72] Both oxygen-sensing and mitochondrial dynamics are disordered in pulmonary-artery smooth-muscle cells, as indicated by normoxic activation of hypoxia-inducible factor 1α (HIF-1α) and mitochondrial fragmentation.[19] The fingerprint of mitochondrial dynamics in pulmonary arterial hypertension is similar to that in cancer, with reduced mitofusin-2–mediated fusion[12] and excessive DRP1-mediated fission.[23]

These acquired defects reflect both transcriptional and post-translational abnormalities. Transcriptional abnormalities include increased HIF-1α

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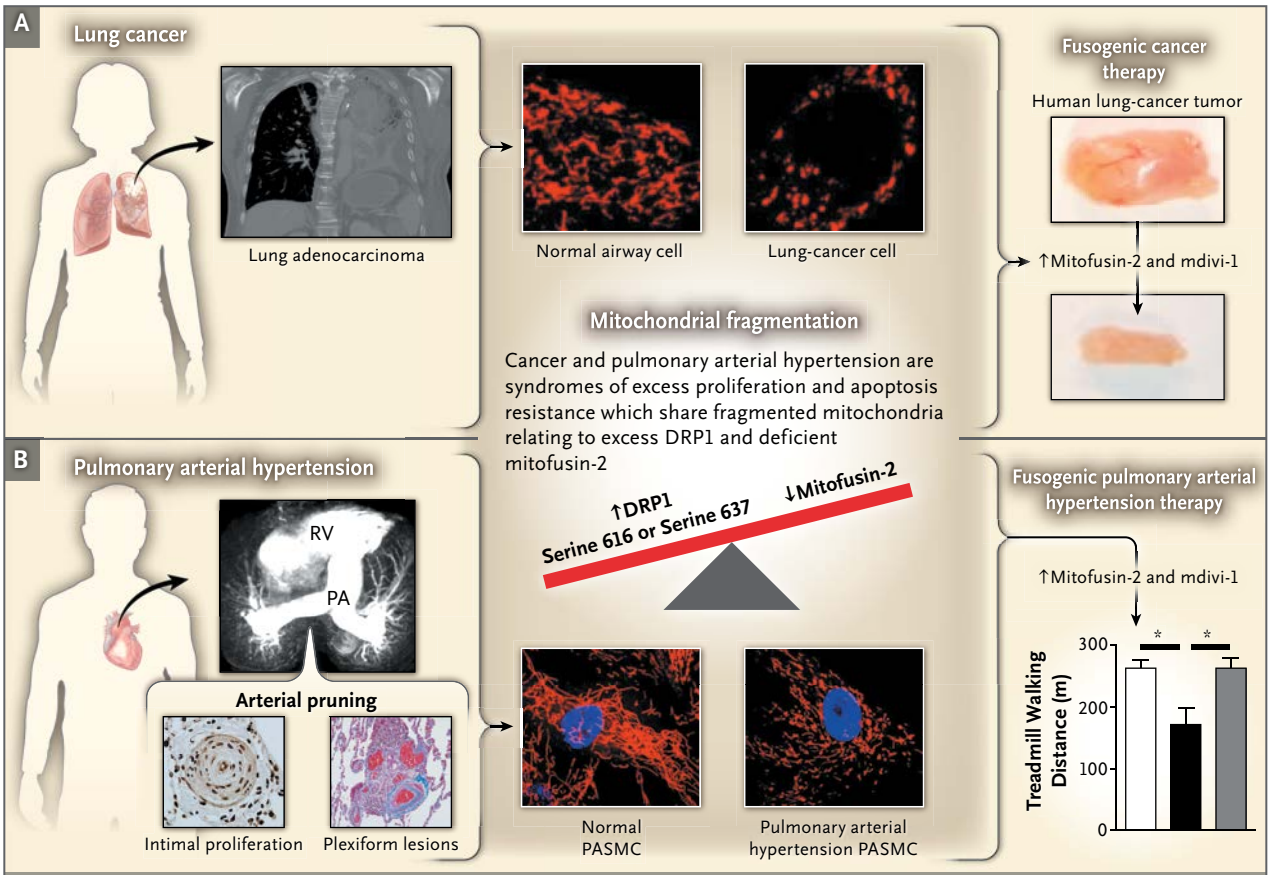
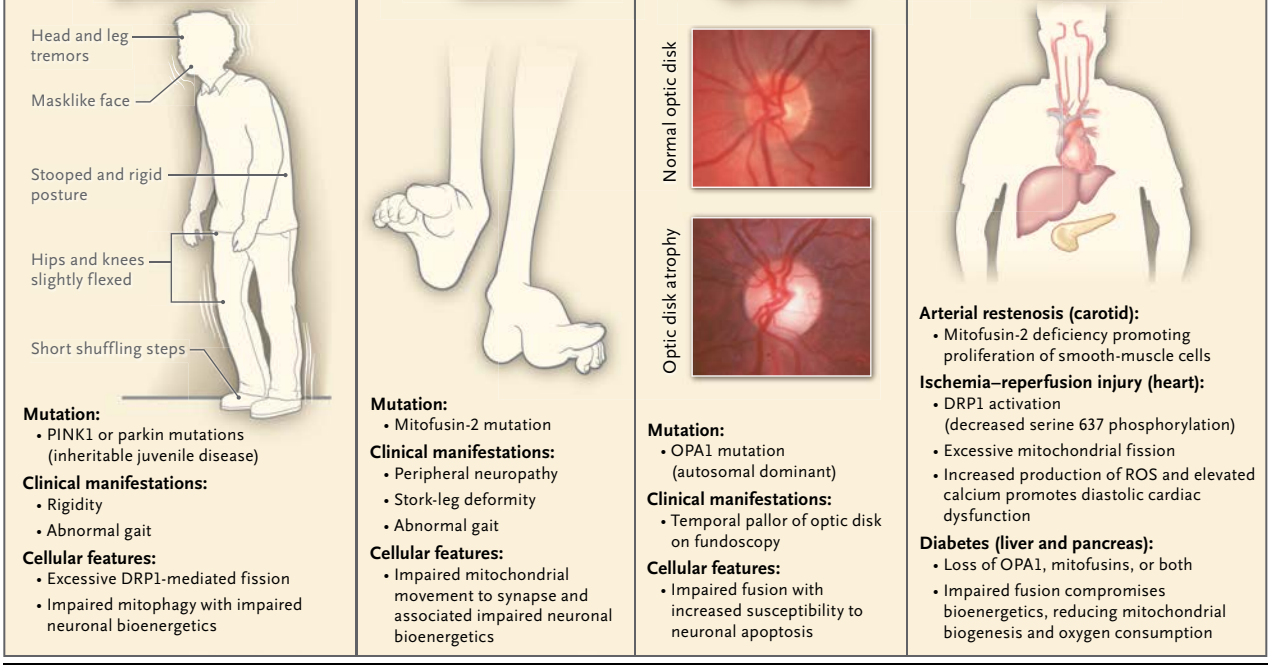


Neurodegenerative and Neuropathic Diseases



Mutation:
- PINK1 or parkin mutations (inheritable juvenile disease)

Clinical manifestations:
- Rigidity
- Abnormal gait

Cellular features:
- Excessive DRP1-mediated fission
- Impaired mitophagy with impaired neuronal bioenergetics

Mutation:
- Mitofusin-2 mutation

Clinical manifestations:
- Peripheral neuropathy
- Stork-leg deformity
- Abnormal gait

Cellular features:
- Impaired mitochondrial movement to synapse and associated impaired neuronal bioenergetics

Mutation:
- OPA1 mutation (autosomal dominant)

Clinical manifestations:
- Temporal pallor of optic disk on fundoscopy

Cellular features:
- Impaired fusion with increased susceptibility to neuronal apoptosis

Arterial restenosis (carotid):
- Mitofusin-2 deficiency promoting proliferation of smooth-muscle cells

Ischemia–reperfusion injury (heart):
- DRP1 activation (decreased serine 637 phosphorylation)
- Excessive mitochondrial fission
- Increased production of ROS and elevated calcium promotes diastolic cardiac dysfunction

Diabetes (liver and pancreas):
- Loss of OPA1, mitofusins, or both
- Impaired fusion compromises bioenergetics, reducing mitochondrial biogenesis and oxygen consumption

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Figure 4 (facing page). Mitochondrial Diseases in Humans.

Fission and fusion imbalances in diseases are characterized by excessive proliferation and resistance to apoptosis. In Panel A, a patient with non–small-cell lung cancer and her chest computed tomographic scan are shown (left). The micrographs (middle) show mitochondrial fragmentation in the cancer cells. This fragmentation results from a fission–fusion mediator profile favoring fragmentation (excess total and activated DRP1 and deficient mitofusin-2). The elevated ratio of serine 616 to serine 637 phosphorylation of DRP1 reflects DRP1 activation and may be a useful cancer biomarker. Mitotic fission is a potential vulnerability of cancer cells and offers many new therapeutic targets. DRP1 inhibition by means of mdivi-1,[8] small inhibitory DRP1 RNA (si-DRP1),[11,66] or adenoviral mitofusin-2 fuses mitochondria, inhibits proliferation, induces apoptosis, and regresses human lung cancers in a xenotransplantation model[11] (right). The mitochondrial fragmentation in cancer and the therapeutic effects of mdivi-1 are shown in Videos 2, 3, and 4, which show a normal airway epithelial cell (Video 2), a lung-cancer cell (Video 3), and a lung-cancer cell treated for 16 hours with mdivi-1 (Video 4). In Panel B, a patient with pulmonary arterial hypertension is shown (left). A magnetic resonance imaging angiogram shows pruning of distal pulmonary arteries. RV denotes right ventricle, and PA pulmonary artery. The loss of pulmonary arteries reflects obstruction by complex arterial lesions (as shown in the histologic specimens). Arteriopathy is due in part to the presence of hyperproliferative, apoptosis-resistant pulmonary-artery smooth-muscle cells (PASMCs) (middle). These cells share with cancer cells the features of mitochondrial fragmentation and fission–fusion imbalance. They also overexpress activated DRP1 and underexpress mitofusin-2. Increased expression of cyclin B1–CDK1 in pulmonary arterial hypertension activates DRP1 by phosphorylating it at serine 616. The fundamental cause of the fragmentation is multifactorial and includes normoxic activation of HIF-1α and a deficiency of PGC-1α. Preventing mitotic fission with the use of mdivi-1 or si-DRP1 causes cell-cycle arrest at the G2–M checkpoint in pulmonary arterial hypertension PASMCs.[11] Despite having doubled their DNA content, PASMCs treated with a DRP1 inhibitor cannot complete mitosis and are removed by means of apoptosis.[23] The mitochondrial fragmentation in pulmonary arterial hypertension and the therapeutic affects of mdivi-1 are shown in Videos 5, 6, and 7, which show a normal smooth-muscle cell (Video 5), a PASMC obtained from a patient with pulmonary arterial hypertension (Video 6), and a PASMC (also obtained from a patient with pulmonary arterial hypertension) treated for 16 hours with mdivi-1 (Video 7). Increased levels of cytosolic calcium in pulmonary arterial hypertension may promote fission by activating calcineurin (which dephosphorylates DRP1 serine 637)[35] and CamK (which activates DRP1 in neurons[34]). Although the calcineurin inhibitor cyclosporine causes experimentally induced pulmonary arterial hypertension to regress,[17] it is not known whether this relates to effects on mitochondrial dynamics. Likewise, it is unknown whether CamK-II, the predominant vascular isoform, activates DRP1. Inhaled adenoviral mitofusin-2 and mdivi-1 enhance exercise performance and partially regress experimentally induced pulmonary arterial hypertension in rats, as judged by exercise capacity (bar graph at right).[12,23] Although the relative prevalence of disordered mitochondrial dynamics and its biologic importance in sporadic neurodegenerative diseases are uncertain, heritable conditions indicate the biologic plausibility that increased fission and decreased fusion can be toxic to neurons. As shown in Panel C, heritable juvenile parkinsonism results from autosomal recessive mutations in PINK1[42] or parkin.[45] The PINK1–parkin pathway normally maintains a fused mitochondrial network,[40] reduces mitochondrial oxidant stress, prevents fission, and maintains normal mitochondrial membrane potential. Preclinical data suggest that strategies that inhibit fission, enhance fusion, or selectively restore mitophagy may have therapeutic benefit in this form of parkinsonism and other neurodegenerative syndromes such as Huntington's disease. As shown in Panel D, Charcot–Marie–Tooth disease is an inherited peripheral neuropathy.[67] There are two types of Charcot–Marie–Tooth disease. Type 1 primarily affects Schwann cells and is associated with slow nerve conduction, whereas type 2 affects motor neurons and is associated with normal nerve conduction. The axonal subtype, type 2A, usually results from autosomal dominant loss-of-function mutations in mitofusin-2. Mitofusins can form homodimers or heterodimers. Mitofusin-1 (but not mitofusin-2) can rescue mutant mitofusin-2; this suggests that variability in mitofusin-1 expression could underlie disease heterogeneity and that modulating neuronal mitofusin-1 expression might have a therapeutic role.[68] Panel E shows autosomal dominant optic atrophy, a heritable form of blindness that results from mutation and loss of function of OPA1. Reproduced from Olichon et al.[14] with the permission of the publisher. Panel F shows cardiometabolic diseases such as arterial restenosis. Mitofusin-2 expression is reduced in models of arterial injury; this contributes to intimal proliferation and restenosis. Mitofusin-2 augmentation might be a useful strategy for reducing restenosis in systemic arteries.[7] Ischemia–reperfusion injury is characterized by increased fission. DRP1 inhibition, whether achieved directly (by administering mdivi-1 or siDRP1) or indirectly (by preventing dephosphorylation of DRP1 serine 637 with the use of therapeutic hypothermia or the calcineurin inhibitor FK506), may be cardioprotective.[36] Impaired fusion due to reduced mitofusin-2 and its transcriptional coactivator PGC-1α may contribute to diabetes and the metabolic syndrome. However, simply augmenting fusion is unlikely to be effective, since excessive fusion can interfere with beneficial mitophagy.

activation[23,31] and decreased PGC-1α activity.[12] Activation of HIF-1α in normal pulmonary-artery smooth-muscle cells is sufficient to cause DRP1-dependent mitochondrial fission.[23] The normoxic HIF-1α activation in pulmonary arterial hypertension leads to a proliferative, apoptosis-resistant milieu through both metabolic effects (pyruvate dehydrogenase inhibition) and induction of a fission–fusion imbalance; the latter mechanism is not well understood.[17]

Post-translational abnormalities (increased cyclin B1–CDK1–dependent and CamK-dependent DRP1 phosphorylation) activate DRP1 and promote fission.[23] Many of these pro-fission, anti-fusion abnormalities occur in models of pulmonary arterial hypertension created in healthy rats and persist in cell culture, suggesting epigenetic underpinnings.[17]

Mitofusin-2 levels are reduced in pulmonary arterial hypertension, contributing to both mitochondrial fragmentation and the proliferative diathesis.[12] Mitofusin-2 was originally named the

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"hyperplasia-suppressor gene"[7] because of its antiproliferative effects on systemic arterial smooth-muscle cells.[7] Mitofusin-2 down-regulation in pulmonary arterial hypertension is associated with low levels of PGC-1α,[12] the transcriptional coactivator of mitofusin-2.[73] Preclinical testing showed that augmenting mitofusin-2 decreases proliferation and increases apoptosis of pulmonary-artery smooth-muscle cells and leads to partial regression of experimentally induced pulmonary arterial hypertension in vivo in rats.[12] Although this therapeutic benefit is associated with restoration of fusion, the link between fusion and inhibition of proliferation is controversial. In systemic arterial cells, truncated mitofusin-2 variants that do not localize to mitochondria retain antiproliferative properties by inhibiting extracellular signal-regulated and mitogen-activated protein kinases.[7]

Patent Ductus Arteriosus

Functional ductus arteriosus closure is initiated by oxygen-dependent vasoconstriction within minutes after birth. An increase in oxygen is accompanied by increases in oxidative metabolism, electron transport chain activity, and generation of reactive oxygen species. Reactive oxygen species are converted by mitochondrial superoxide dismutase 2 (SOD2) to hydrogen peroxide, a diffusible oxidant messenger that regulates ion channels and enzymes, thereby initiating ductal constriction.[56] A subsequent fibrogenic, proliferative mechanism results in anatomical closure.

Recently, it was discovered that the redox-sensing mechanism of the human ductus arteriosus relies on mitochondrial fission. Within 5 minutes after an increase in oxygen, DRP1-mediated fission fragments ductus arteriosus smooth-muscle cell mitochondria.[10] Inhibiting DRP1 selectively prevents oxygen-induced ductal constriction. Oxygen induces fission by cyclin B1–CDK1–dependent and CamK-dependent, post-translational DRP1 activation.[10] In ductus arteriosus smooth-muscle cells, fission activates pyruvate dehydrogenase and increases electron transport chain complex 1 activity, creating an oxidant environment. Conversely, inhibiting fission interrupts redox signaling, preventing oxygen-dependent production of mitochondrial-derived hydrogen peroxide. This link between structure and electron transport chain assembly and activity is similar to an observation made in drosophila, in which a knockout of PINK1 (phosphatase and tensin homologue–induced putative kinase 1) impairs fission and reduces bioenergetic capacity by causing defective electron transport chain complex assembly.[74] Although sustained DRP1 inhibition prevents anatomical closure in an ex vivo human ductus arteriosus model, it is not known whether impaired mitochondrial fission contributes to spontaneous patent ductus arteriosus.

NEURODEGENERATIVE DISEASES

Neuronal development requires both DRP1[75] and mitofusin-2.[76] Neuronal DRP1 knockout causes mitochondrial maldistribution, apoptosis, and perinatal death due to brain hypoplasia (Fig. 4C, 4D, and 4E).[75] The beneficial role of DRP1-induced fission in human brain development is highlighted by the description of an infant with a dominant-negative DRP1 mutation.[16] She had hypotonia, microcephaly, and optic atrophy and died suddenly at 37 days of age; studies showed impaired mitochondrial metabolism and hyperfusion, which were consistent with impaired fission.[16] Although some level of fission is required for brain development, increased fission and impaired fusion, resulting in mitochondrial fragmentation, are the rule in adult neurodegenerative diseases.

Familial Parkinsonism

Parkinson's disease is a neurodegenerative syndrome characterized by resting tremor, rigidity, and bradykinesia, resulting from death of dopaminergic neurons in the substantia nigra. Although most cases of parkinsonism are sporadic, rare heritable, juvenile cases result from autosomal recessive mutations in mitochondrial-targeted kinases PINK1[58] and parkin[59] (Table 1 and Fig. 4C).

PINK1, a serine–threonine kinase, protects neurons by preventing stress-induced mitochondrial depolarization and apoptosis[42] and reducing mitochondrial oxidative stress and fission.[39] PINK1 also phosphorylates parkin, targeting it to depolarized mitochondria and thereby enhancing mitophagy.[77] Parkin, a ubiquitin E3 ligase, protects neurons by ubiquitinating proteins that are abnormal in structure or quantity (including DRP1[46]), marking them for proteosomal degradation. Parkin also ubiquitinates dysfunctional mitochondria, leading to their removal by mitophagy.[78] PINK1 and parkin loss-of-function mutations have complex effects; however, increased DRP1-mediated mitochondrial fission is an important consequence and contributes to neuro-

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nal death.[46,79,80] Neurons lacking PINK1 or parkin accumulate DRP1, and the resulting excessive fission increases oxidative stress and reduces ATP production.[39,40,81] These neurons can be rescued by inhibiting fission with the use of mdivi-1[82] or by enhancing fusion through augmentation of mitofusin-2 or OPA1.[39,40,81] Excessive fission is not neuronally restricted; it is detected in dermal fibroblasts from patients.[41] How this relatively generalized abnormality of mitochondrial dynamics results in tissue-restricted disease is an unresolved mystery that is relevant to optic atrophy and Charcot–Marie–Tooth disease type 2A.

PINK1 and parkin also regulate mitophagy. The relationship between mitochondrial dynamics and mitophagy is uncertain; however, the autophagic machinery both delivers mitochondria to lysosomes and contributes to mitochondrial fragmentation[39] (Fig. 3A). At physiologic levels, both mitochondrial fission and mitophagy are beneficial, working together to eliminate damaged and depolarized mitochondria[39]; however, an excess of either process leads to cell death.

Two pesticides that promote parkinsonism (the electron transport chain complex 1 inhibitor rotenone[83] and paraquat[84]) induce mitochondrial fragmentation. Rotenone and paraquat initiate parkin-mediated mitochondrial depolarization. They then trigger fragmentation by promoting mitofusin degradation, through a proteosomal mechanism mediated by the AAA+ superfamily of ATPases and valosin-containing protein p97.[47] The AAA-family ATPases interact with p97 to compose a ubiquitin–proteosome pathway that extracts proteins such as mitofusin-2 from the outer mitochondrial membrane into the cytosol, where they ultimately undergo proteosomal degradation. Removing mitofusins from the outer mitochondrial membrane inhibits fusion and prevents abnormal mitochondria from reintegrating into the network, thereby facilitating mitophagy.[47] This so-called outer mitochondrial membrane–associated degradation pathway[47] mediates mitochondrial quality control and is analogous to the endoplasmic reticulum quality-control pathway (ERAD).[85]

Alzheimer's Disease

The most common cause of dementia is Alzheimer's disease, which is characterized by accumulation of amyloid plaques, neurofibrillary tangles, and loss of connections between neurons. In one study, β-amyloid increased neuronal nitric oxide, generating S-nitrosylated, activated DRP1, which caused fission and neuronal damage.[86] Inhibition of DRP1 nitrosylation reduced neurotoxicity. However, a subsequent investigation could neither replicate the effects of S-nitrosylation on DRP1 activity nor support a role for this mechanism in Alzheimer's disease.[87]

Huntington's Disease

An autosomal dominant disease characterized by choreoathetosis, dementia, and premature death, Huntington's disease results from expansion of a CAG trinucleotide DNA sequence encoding a polyglutamine tract in the huntingtin protein.[88] The polyglutamine accumulation causes protein misfolding and promotes abnormal protein interactions. Mutant huntingtin interacts with and activates DRP1, increasing fission and neuronal sensitivity to apoptosis in rat neurons and in fibroblasts from patients with Huntington's disease.[20] The severity of CAG expansion in the huntingtin gene, an established predictor of disease severity, also predicts the severity of fission.[88] The importance of polyglutamine-containing proteins for fission and cell death is recapitulated in cellular and *Caenorhabditis elegans* models of Huntington's disease.[88] In both models, inhibiting DRP1 or augmenting mitofusin-2 restores mitochondrial fusion, preserves ATP, and prevents cell death.[88]

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a motor neuron disease that causes weakness and death. In a rare heritable form of the disease, mutation of superoxide dismutase 1 causes DRP1-mediated fission, which inhibits axonal transport of mitochondria, causing neuronal death.[89] The mitochondrial matrix sirtuin SIRT3 and PGC-1α provide protection against excessive fission and neuronal death.

NEUROPATHIES

Charcot–Marie–Tooth Disease

An inherited peripheral neuropathy, Charcot–Marie–Tooth disease (Fig. 4D) usually results in atrophy of calves, foot deformities, and abnormal gait. The axonal subtype of Charcot–Marie–Tooth disease, type 2A, is generally due to autosomal dominant, missense mutations in the fusion-mediating, coiled-coil, and GTPase domains of mitofusin-2.[8,90] Mito-

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fusin-2 mutations inhibit metabolism, causing energetic deprivation that limits mitochondrial mobility to synapses.[61] A single mitofusin-2 mutation can result in a heterogeneous disease phenotype and an onset of disease at various ages within one family.[90]

Optic Atrophy

Mutations in *OPA1* cause an autosomal dominantly inherited optic neuropathy[14] (Fig. 4E). Affected patients have progressive loss of visual acuity that often leads to blindness by the second decade of life.[91] The loss of OPA1-mediated fusion leaves fission unopposed; this predisposes retinal ganglion cells to apoptosis, ultimately resulting in optic-nerve degeneration.[91] Most of the approximately 96 known mutations occur in the GTPase domain of *OPA1* or its 3′ terminus, resulting in dominant-negative and haploinsufficiency phenotypes, respectively.[67] A mitofusin-2 mutation can also result in optic atrophy, a reminder of the interdependence of these fusion pathways.[92]

CARDIOMETABOLIC DISEASES

Diabetes Mellitus

Mitochondrial fusion defects promote diabetes in murine models of type 1 diabetes mellitus. In patients with obesity or type 2 diabetes mellitus (Fig. 4F), fusion is also impaired, as evidenced by reduced mitofusin-2 expression and decreased mitochondrial size.[30] Hepatic deletion of mitofusin-2 increases reactive oxygen species generation, impairs insulin signaling, and causes glucose intolerance.[93] Down-regulation of mitofusin-2 impairs oxygen consumption and promotes diabetes. Mice with pancreatic β-cell–specific OPA1 knockout also have impaired glucose-stimulated insulin secretion, and hyperglycemia develops in such mice.[94] Although severe impairment of fusion promotes diabetes, tonic restoration of fusion is unlikely to be a simple remedy because some mitochondrial fragmentation is required for mitophagy. Mitochondria undergo asymmetric fission, resulting in both normal mitochondria and depolarized, dysfunctional mitochondria that are deficient in OPA1.[95] OPA1 deficiency isolates abnormal mitochondria, preventing network reintegration. This localized impairment of fusion and mitophagy is beneficial. Indeed, inhibiting mitophagy by enhancing fusion (overexpressing OPA1) leads to accumulation of damaged mitochondria with impaired respiration and reduced insulin secretion.[95]

Ischemia–Reperfusion Injury

Despite effective resuscitation techniques, mortality from cardiac arrest remains high, in part because of ischemia–reperfusion injury (Fig. 4F). In the kidney, mdivi-1 protects renal tubular cells from fission induced by ischemia–reperfusion injury[96]; it is also protective in a cardiac ischemia–reperfusion injury model.[97] During cardiac arrest, DRP1 is activated by calcineurin-mediated dephosphorylation of DRP1 at serine 637.[36] The resulting fission increases reactive oxygen species production, increases levels of calcium, and impairs diastolic relaxation. DRP1 inhibition, whether achieved directly or by targeting calcineurin, may have a therapeutic effect (Fig. 4F).

Cardiomyopathy

Because germline mitofusin knockouts are lethal at the embryonic stage, tissue-specific, conditional knockout mice are required to study the role of mitofusins in the heart. Conditional cardiac ablation of both mitofusin isoforms simultaneously has deleterious effects on mitochondrial morphologic features, respiration, and contractile function, resulting in death from cardiac failure.[98,99] Conversely, deletion of only one mitofusin isoform is cardioprotective against ischemia–reperfusion injury and reactive oxygen species. Conditional cardiac deletion of mitofusin-2 results in mitochondrial dysfunction and hypertrophy, both of which are mild, with a slight reduction in left ventricular function. However, these mice have reduced susceptibility to activation of the mitochondrial permeability transition pore.[100] The same group of investigators subsequently showed that conditional mitofusin-1 knockouts also have normal baseline cardiac function and are protected from reactive oxygen species–induced cardiotoxicity.

FUTURE DIRECTIONS

Mitochondrial dynamics are involved in the mechanisms of a variety of human diseases and may offer therapeutic targets. Before attempts are made to manipulate mitochondrial dynamics therapeutically, further evaluation is required to identify the optimal molecular targets and define safe and effective doses of fission and fusion modulators that selectively target the relevant cellular populations. Additional pharmacologic or molecular modulators of fission and fusion are needed. Qi et al.[101] recently described a small, rationally designed peptide inhibitor (P110) that

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prevents DRP1 activation and fission by interfering with the protein–protein interaction between DRP1 and its mitochondrial adaptor target protein, Fis1. Validation of the use of peripheral cells (i.e., fibroblasts) to assess fission and fusion and metabolism is required. The usefulness of measuring mediators of mitochondrial dynamics in peripheral blood as disease biomarkers merits study. Future research directions are highlighted in Table 1 in the Supplementary Appendix.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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