REVIEW

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Mitochondrial disorders as windows into an ancient organelle

Scott B. Vafai[1,2] & Vamsi K. Mootha[1,2,3]

Much of our current knowledge about mitochondria has come from studying patients who have respiratory chain disorders. These disorders comprise a large collection of individually rare syndromes, each presenting in a unique and often devastating way. In recent years, there has been great progress in defining their genetic basis, but we still know little about the cascade of events that gives rise to such diverse pathology. Here, we review these disorders and explore them in the context of a contemporary understanding of mitochondrial evolution, biochemistry and genetics. Fully deciphering their pathogenesis is a challenging next step that will inspire the development of drug treatments for rare and common diseases.

The field of mitochondrial medicine began in 1959 when Swedish endocrinologist Rolf Luft and his colleagues described the case of a young woman with euthyroid hypermetabolism, which was characterized by profuse sweating and weight loss despite high calorie intake[1]. Muscle biopsy and enzyme analysis — which are now a linchpin for the diagnosis of these disorders — revealed an uncoupling of mitochondria in the patient. Although described decades ago, the condition has been reported only once more[2] and the root cause of Luft's disease remains a mystery.

Since the initial case report, more than 150 distinct genetic mitochondrial syndromes have been defined. The largest subset arises from lesions that influence the function of the respiratory chain, which affect at least 1 in 5,000 live births[3] and are the focus of this Review. These diseases can present either in infancy or adulthood, and in a multisystemic or highly tissue-specific manner. Signature traits can include lactic acidosis, skeletal myopathy, deafness, blindness, subacute neurodegeneration, intestinal dysmotility and peripheral neuropathy. Most organ systems can be affected or spared, in varying combinations (Fig. 1).

The clinical features of mitochondrial disorders have been reviewed in detail[4,5], but select examples are illustrative. Leber's hereditary optic neuropathy (LHON) — which is the result of point mutations in a ubiquitously expressed mitochondrial DNA (mtDNA)-encoded respiratory chain protein — demonstrates remarkable tissue specificity, with patients developing sudden vision loss as young adults. By contrast, point mutations in an mtDNA-encoded transfer RNA cause mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), with a multisystem presentation that includes seizures, impaired hearing, stroke-like episodes and lactic acidosis. Mutations in the nuclear-encoded mitochondrial DNA polymerase gene *POLG* underscore pleiotropy: some mutations result in mild ocular-muscle weakness, whereas others produce Alpers' syndrome (characterized by psychomotor regression, seizures and liver failure). These genetic disorders can be mimicked by drugs that have off-target toxicity. A 1995 phase 2 clinical trial for fialuridine — a promising new antiviral for hepatitis B — was halted after the drug caused lactic acidosis, myopathy, neuropathy and even fatal liver failure in some individuals[6].

As our ability to define the genetic and environmental bases of mitochondrial disorders has accelerated, new questions have arisen. Why are some disorders highly tissue-specific, and others multisystemic? How can loss-of-function mutations in bacteria-conserved proteins even be compatible with life? Why do some drugs that inhibit mitochondria cause disease, whereas other inhibitors are protective against it? What underlies sporadic cases of mitochondrial disease? The answers to most of these questions are a mystery, but if solved they could provide fundamental insight into metabolism, lead to new therapies for orphan diseases, and help us to rigorously evaluate the role of mitochondria in common diseases.

In this Review, we outline four of the key properties of mitochondria — their evolutionary origins, metabolic interconnections, heterogeneity and robustness — that help to explain some of the mysterious features of these disorders. We review recent insight into their genetic architecture, and emerging themes in their pathogenesis. Finally, we discuss the challenges and opportunities that lie ahead for this relatively young field of medicine.

Origin and evolution of mitochondria

Mitochondria have an endosymbiotic origin and retain many vestiges of their bacterial ancestry, including a double membrane and a circular genome (the mtDNA). They resemble microbes in that they are typically about one micrometre in scale and constantly move, divide and fuse to form a dynamic network. Although mitochondria are referred to as semi-autonomous organelles, billions of years of expansive and reductive evolution (Fig. 2a) — accompanied by transfer of most of their genes to the nuclear genome — have now effectively hard-wired these organelles within eukaryotic cells.

Human mtDNA is maternally inherited and encodes only 13 proteins, as well as the 22 tRNA and 2 ribosomal RNA genes required for their translation. All other proteins required to maintain and express mtDNA are encoded by the nuclear genome. Great progress has been made in defining the mammalian mitochondrial proteome, with more than 1,100 proteins assigned to this compartment[7]. Interestingly, mtDNA has a monophyletic origin[8], whereas the history of the mitochondrial proteome is far more complex[7,9]. Of the 1,100 known mitochondrial proteins, about two-thirds have bacterial origins (probably from multiple phyla), with the rest representing

[1]Departments of Molecular Biology and Medicine, Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [2]Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, USA. [3]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.

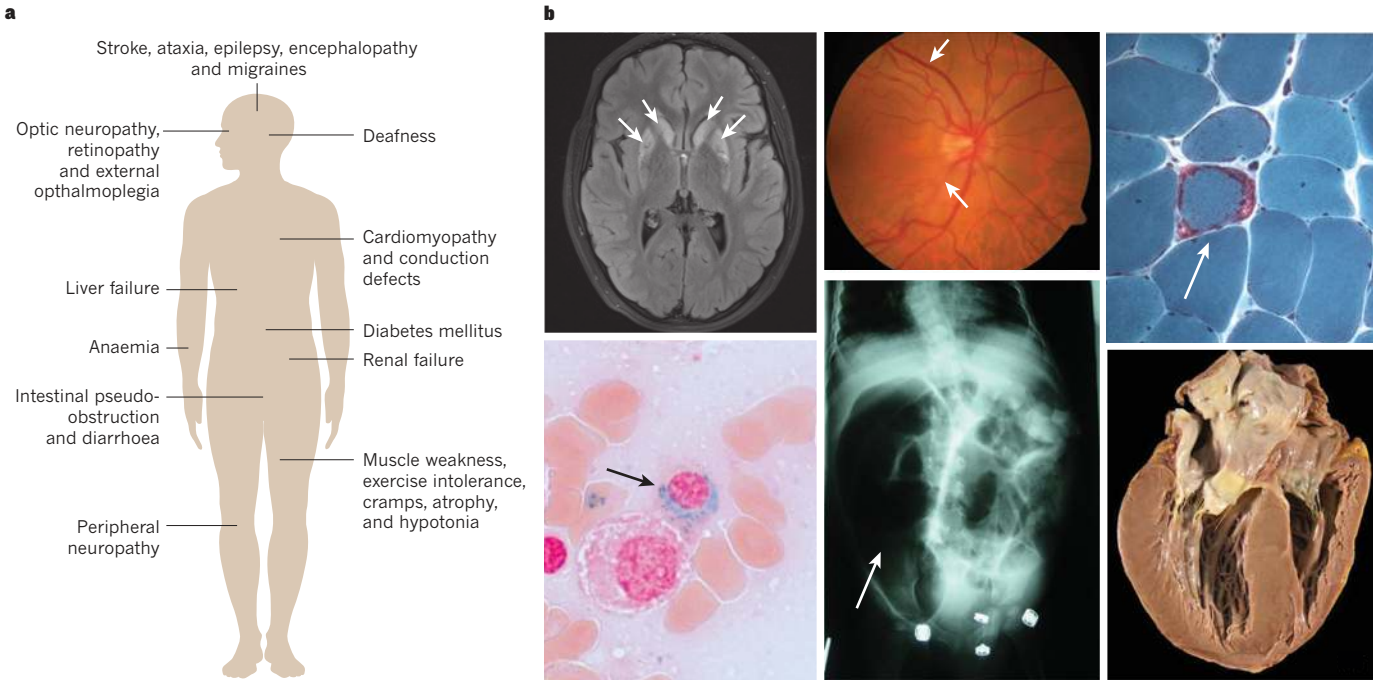

Figure 1 | Phenotypic spectrum of mitochondrial disorders. **a**, Common clinical manifestations of mitochondrial disorders. **b**, Clinical images depicting pathology from patients with a variety of mitochondrial disorders. Clockwise from top left, 3-Tesla fluid-attenuated inversion-recovery brain magnetic resonance imaging demonstrating Leigh syndrome lesions, which are characterized in this image by a hyperintense signal within the caudate and putamen bilaterally (arrows) seen on an axial cut through the basal ganglia; retinal image of the acute phase of Leber's hereditary optic neuropathy, demonstrating an optic disc with swollen nerve fibre layer that is associated with engorged and obscured blood vessels (arrows); ragged red fibre (arrow) seen on a modified Gomori-trichrome-stained skeletal-muscle section; anterior four-chamber cross-section of a heart that shows signs of hypertrophic cardiomyopathy, including cardiomegaly and asymmetrical septal hypertrophy; plain abdominal radiograph, showing massive bowel distention (arrow) in the setting of chronic intestinal pseudo-obstruction without evidence of mechanical obstruction; and bone-marrow aspirate sample that has been stained for iron to demonstrate a ringed sideroblast (arrow) — characterized by a halo of iron-laden mitochondria around the nucleus of an erythrocyte precursor — from a patient with myopathy, lactic acidosis and sideroblastic anaemia syndrome.

eukaryotic innovations[7]. The mosaic composition of human mitochondria is evident in the organelle's replication and translation machinery, with the ribosome closely resembling its bacterial counterpart[10] and the DNA polymerase resembling that of a viral (bacteriophage) ancestor[11]. As discussed later, the molecular basis of certain mitochondrial pathologies becomes clear when the ancestry of the organelle is taken into account.

During the course of evolution, the organelle ceded ownership of certain pathways to the rest of the cell, but retained and even acquired others. For example, ribonucleotide reductase — which is used for *de novo* synthesis of deoxyribonucleotides — is found only in the cytosol, and deficiency of this enzyme causes mtDNA depletion syndrome[12]. In other cases, the mitochondria have retained a duplicated copy of the cytosolic pathway, such as for tetrahydrofolate-dependent one-carbon metabolism[13]. These paralogous one-carbon pathways seem to have adopted a different functional importance, and may be particularly relevant in disease states such as cancer[14]. Understanding the logic of compartmentalization and paralogous pathways is an ongoing challenge.

Respiratory chain and its connections

At the heart of mitochondria is the respiratory chain, the core machinery for oxidative phosphorylation (Fig. 2b). Classically, the respiratory chain is defined as four macromolecular complexes that catalyse electron transfer from reducing equivalents, which are derived from intermediary metabolism, to molecular oxygen. Free energy is conserved by coupling electron transport to the formation of a proton gradient, or proton motive force (PMF), by three of these complexes (I, III and IV), which is then dissipated by F_1F_0-ATPase (complex V) for ATP synthesis. These complexes are associated with the inner membrane and consist of about 90 protein components, only 13 of which are mtDNA-encoded. Although typically depicted as a linear chain operating in isolation, the respiratory chain is truly a hub in the network of cellular metabolism that is characterized by convergence and divergence of pathways, supercomplex formation and reversibility.

Almost all of the cell's redox reactions ultimately feed into the respiratory chain (Fig. 2b). Complexes I and II mediate two-electron transfer from NADH and $FADH_2$, respectively, to the mobile electron carrier coenzyme Q, providing links to the tricarboxylic acid (TCA) cycle. Coenzyme Q can also receive electrons from *de novo* pyrimidine biosynthesis, fatty-acid and amino-acid oxidation, choline oxidation (ultimately affecting one-carbon metabolism), and glycolysis. Complex III, through its 'Q-cycle', is an adaptor that receives two electrons from reduced coenzyme Q and funnels individual electrons to cytochrome *c*. Complex IV ends the respiratory chain by accepting electrons from cytochrome *c* and using them to fully reduce oxygen to water. Reactive oxygen species (ROS) are potentially toxic by-products of these reactions — especially at complexes I and III — but are buffered by dedicated superoxide dismutase and catalase, as well as glutathione, thioredoxin and protein thiol systems. Interruptions to the respiratory chain can therefore affect nucleotide pools, TCA-cycle flux, one-carbon metabolism and ROS signalling to unleash numerous ripples (discussed later).

The PMF is best known for driving ATP synthesis through oxidative phosphorylation, but it is linked to many other processes (Fig. 2b). The nicotinamide nucleotide transhydrogenase relies on the PMF to regenerate mitochondrial NADPH, which is required for ROS homeostasis. Furthermore, the PMF is coupled to solute and ion transport across the inner membrane, and collapse of the PMF can halt essential biosynthetic reactions, such as Fe–S cluster

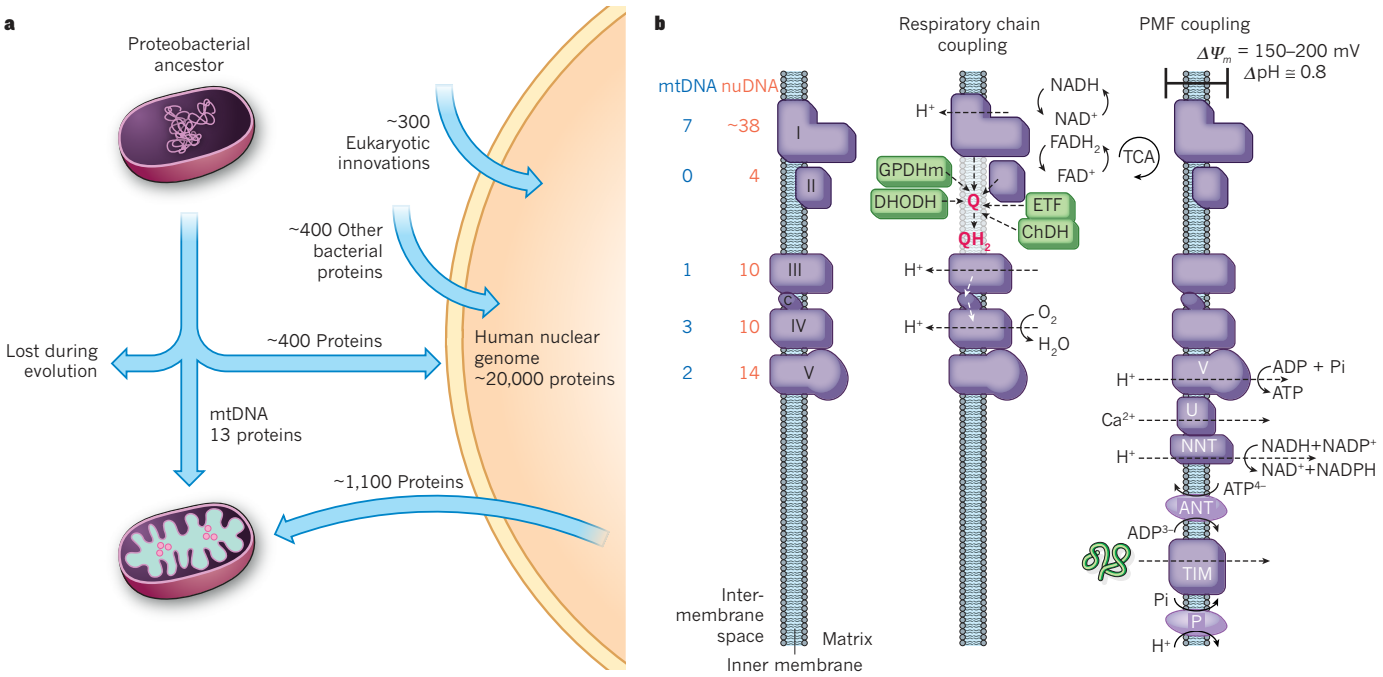

Figure 2 | Mitochondrial evolution and the respiratory chain. **a**, The modern human mitochondrial proteome consists of 13 proteins, which are encoded by mitochondrial DNA (mtDNA) and are a vestige of the original proteobacterial genome, as well as at least 1,100 additional proteins that are known to be encoded by the nuclear genome (nuDNA). Of these proteins, about 400 have a proteobacterial origin, determined by sequence similarity to the closest living relative of the ancestral proteobacterial species, *Rickettsia prowazekii*[7,8], with the remaining ancestral proteins lost during evolution. About 400 proteins were obtained from other bacterial organisms — estimated by determining the number of mitochondrial proteins with homologues in other prokaryotic organisms. About 300 proteins have no homologue in any prokaryotic organisms, and are eukaryotic innovations. **b**, On the left is the classic view of complexes I to V, with the number of mtDNA and nuclear-DNA-encoded subunits indicated. In the centre are selected biochemical pathways that are coupled to electron flow through the respiratory chain. Complex I and II transfer electrons from NADH and $FADH_2$, respectively, to coenzyme Q, providing a link with the tricarboxylic acid (TCA) cycle. Coenzyme Q additionally receives reducing equivalents from glycolysis through mitochondrial glycerol-3-phosphate dehydrogenase ($GPDH_m$), *de novo* pyrimidine biosynthesis through dihydroorotate dehydrogenase (DHODH), choline oxidation and one-carbon metabolism through choline dehydrogenase (ChDH), and fatty acid and amino acid oxidation through electron-transferring flavoprotein dehydrogenase (ETF). On the right are selected processes that are coupled to the proton motive force (PMF), including ATP generation through complex V, calcium transport through the uniporter (U), NADPH generation through nicotinamide nucleotide transhydrogenase (NNT), ATP/ADP exchange through the adenine nucleotide translocator (ANT), protein import through the translocase of the inner mitochondrial membrane (TIM). Inorganic phosphate (Pi) transport is through its carrier (P). $\Delta\Psi_m$, mitochondrial membrane potential.

biogenesis and protein import. The importance of the PMF is exemplified by the fact that glycolytic ATP can be consumed by complex V that is run in reverse to defend PMF during states of respiratory chain inhibition.

Although respiratory chain complexes are commonly depicted as freely moving through a random-collision model[15], there is growing evidence to support a solid-state model[16], in which individual complexes are physically grouped into supercomplexes. Native gel separations have demonstrated interactions between complexes I, III and IV[17–19]. In principle, such associations may promote stability and substrate channelling while minimizing ROS formation. The recent identification of genetic factors that are required for supercomplex assembly (reviewed in ref. 20) will allow a rigorous evaluation of their role in disease.

Comparative analysis across species suggests that many pathways connected to the respiratory chain are still undiscovered. For example, human complex I consists of about 45 subunits, including 14 core catalytic subunits that are found in bacterial complex I. Recently, the crystal structures of complex I have been elucidated for *Escherichia coli*[21] and *Yarrowia lipolytica*[22]. The putative mechanism raised by both structures indicates that the known functions, electron transfer and proton pumping, are accomplished by the 14 ancient subunits. What functions do the remaining subunits serve? Although the leading hypothesis is that they are required for assembly, stability or regulation, it is tempting to speculate that complex I may be a scaffolding for additional enzymatic activities. For example, one subunit that is associated with complex I, NDUFAB1, has an acyl carrier protein domain that participates in type II fatty-acid synthesis[23]; and the assembly factor ACAD9 shares sequence homology with very-long-chain fatty-acid dehydrogenase[24]. Moreover, one subset of complex I shares a phylogenomic signature with enzymes that are related to branched-chain amino-acid oxidation[7]. The other potential moonlighting roles that complex I may have require further investigation and could help us to understand the pleiotropic features that are observed in patients.

Mitochondrial heterogeneity

The extent to which mitochondria are specialized within each cellular context is underappreciated. Mitochondria from different organs exhibit distinct patterns of fuel use and biosynthetic capacities. For example, skeletal-muscle mitochondria are adept at oxidizing fatty acids, and brain mitochondria are capable of oxidizing ketones, whereas adrenal mitochondria have a high capacity for steroid hormone biosynthesis. Electron microscopy studies have shown that mitochondrial content and morphology can be highly variable across tissues (Fig. 3a), owing to changes that occur in development and in response to environmental cues. Even within individual cells, mitochondria can exhibit heterogeneity: intermyofibrillar and subsarcolemmal mitochondria within skeletal muscle have distinct fuel preferences and respond differentially to physiological and pathological inputs[25,26]. In fact, one hypothesis postulates that mtDNA has persisted in evolution to endow individual mitochondria with

the ability to tune their local energetic and redox state through local control of gene expression, the so-called co-location for redox regulation hypothesis[27].

Recent proteomic surveys of mitochondria from different organs have quantified the level of molecular heterogeneity, revealing that mitochondria from two distinct organs will typically share about 75% of their components[7]. This tissue heterogeneity has a wide range of functional consequences[7,28]. For example, whereas most respiratory chain complexes are invariant across organs, complex IV exhibits tissue-specific isoforms[7] that were previously posited as crucial for responding to local oxygen tension[29]. The mitochondrial ribosome is used to translate the 13 mtDNA-encoded respiratory chain subunits, all of which are essential and found in all tissues. However, the protein composition of the mitochondrial ribosome exhibits striking tissue diversity[7], the consequences of which are currently unknown. It is tempting to speculate that this variability contributes to the tissue-specific effect of certain mtDNA mutations.

An exciting new frontier in mitochondrial biology is defining the regulatory mechanisms that give rise to the observed heterogeneity, both within cells and across tissues (Fig. 3b). These mechanisms can be grouped into four broad categories, each of which has been reviewed elsewhere: organelle biogenesis[30,31], movement[32], fusion and fission[33], and mitophagy[34]. Co-location for redox regulation may in principle contribute to intracellular heterogeneity, although this hypothesis is in need of rigorous testing, and an understanding of the molecular mechanisms is still lacking. Mutations that affect some of these regulatory programs have already been identified as contributors to disease. For example, mutations in *MFN2* and *OPA1*, both of which are required for mitochondrial fusion, cause Charcot–Marie–Tooth disease type 2A and autosomal dominant optic atrophy, respectively. PINK1 and parkin are required for mitophagy, and are mutated in Mendelian forms of Parkinson's disease.

Robustness

The mitochondrial respiratory chain is a robust system, which is capable of responding to fluctuating nutrient availability and demands. Cardiac mitochondria provide a striking example of robustness: they are capable of maintaining a constant ATP to ADP ratio over a fivefold dynamic range in workload *in vivo* during exercise[35]. Two mechanisms underlying this regulation have been identified. First, Chance and Williams[36], in their pioneering studies of isolated mitochondria, demonstrated that under many experimental conditions the rate of respiration and ATP synthesis is largely controlled by the availability of ADP — a feedback mechanism they termed respiratory control. The second mechanism posits that calcium operates in a feed-forward manner to ensure matched ATP use in the cytosol and its production in mitochondria[37]. Many cytosolic processes, such as neurotransmission and muscle contraction, are triggered by a rise in cytosolic calcium, and the same calcium signal can be transmitted into the matrix through the uniporter to stimulate the TCA cycle to ensure ATP production.

Robustness probably extends across the entire respiratory chain and its many coupled reactions, as evidenced by classic studies of metabolic control analysis (MCA)[38,39]. MCA provides an experimental and theoretical framework with which to understand the distribution of flux control of a system property. For a system flux J, the control coefficient C^J_{Ei} exerted by enzyme E_i is defined as $(\Delta J/J)/(\Delta E_i/E_i)$, in which $\Sigma C^J_{Ei} = 1$. C^J_{Ei} denotes the percentage of control exerted by a single enzyme on the system. Experimentally, small molecules can be used to modulate E_i while J is measured. MCA has been used[40] to show that control of respiration is broadly distributed and, moreover, that the distribution of control depends on workload. An important implication of such work is that the activity of a respiratory chain complex can be varied over a wide regime before overall respiration is affected (Fig. 3c).

Robustness of mitochondrial metabolism has important implications for understanding disease[41]. First, the robustness may be the reason why

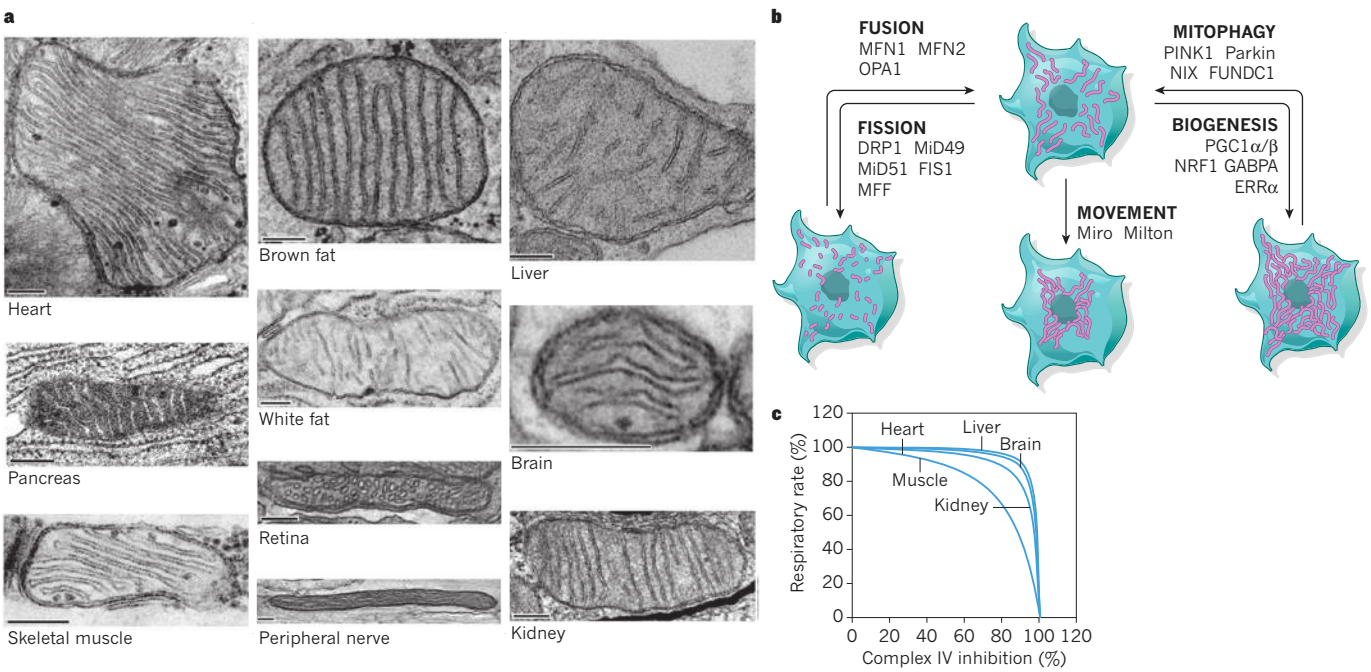

Figure 3 | Mitochondrial tissue heterogeneity and robustness. **a**, Electron micrographs of mitochondria from various tissues, highlighting the diversity across tissues (scale bar, 200 nm). **b**, Processes that give rise to mitochondrial heterogeneity across tissues and within cells: movement, biogenesis, mitophagy, fusion–fission. Key regulators of these processes are listed. DRP1 encoded by *DNM1L*; ERRα encoded by *ESRRA*; FIS1 encoded by *FIS1*; FUNDC1 encoded by *FUNDC1*; GABPA encoded by *GABPA*; MFF encoded by *MFF*; MFN1 encoded by *MFN1*; MFN2 encoded by *MFN2*; MiD49 encoded by *SMCR7*; MiD51 encoded by *SMCR7L*; Milton, which represents the Milton family of proteins encoded by *TRAK1* and *TRAK2* in humans; Miro, which represents the MIRO family of proteins, encoded by *RHOT1* and *RHOT2* in humans; NIX encoded by *BNIP3L*; NRF1 encoded by *NRF1*; OPA1 encoded by *OPA1*; Parkin encoded by *PARK2*; PGC1α encoded by *PPARGC1A*; PGC1β encoded by *PPARGC1B*; and PINK1 encoded by *PINK1*. **c**, Respiratory rate in isolated mitochondria from five types of rat tissue with complex IV inhibited to varying degrees with KCN. The rate is expressed as a percentage of the respiratory rate observed in untreated mitochondria from that tissue. Panel **c** adapted with permission from ref. 42.

even severe mutations can be tolerated within the oxidative phosphorylation system and be compatible with life. Robustness of mitochondria implies that the system will be tolerant of hypomorphic alleles, which may be genetic contributors to disease when compounded with the appropriate stress, environmental modifier or a second genetic hit. The control coefficients of different respiratory chain complexes have been shown[42] to vary across rat tissues (Fig. 3c), leading to speculation that tissue-specific control coefficients have an influence when pathology becomes manifest. However, this attractive hypothesis still needs formal proof.

Genetics of mitochondrial disorders

Over the past 25 years, studies of individual patients and families who are affected by mitochondrial disorders have yielded a wealth of insight into their genetic architecture. These genetic lesions can lie in the mtDNA or nuclear DNA and have revealed the pathways that support respiratory chain assembly and activity (Fig. 4 and Table 1). These genetic studies have also helped to expand the phenotypic spectrum of mitochondrial disorders.

Mutations in the mtDNA

The sequencing of human mtDNA in 1981 (ref. 43) launched the molecular era of mitochondrial medicine. Two landmark papers in 1988 (refs 44 and 45) reported point mutations and deletions in mtDNA in LHON and mitochondrial myopathy, respectively. Since then, more than 300 point mutations, deletions and duplications have been associated with a wide variety of symptoms (http://www.mitomap.org). mtDNA is oocyte-derived, so inherited forms of these disorders follow maternal inheritance. Although some mtDNA disorders (such as LHON) are homoplasmic, others (such as MELAS) are heteroplasmic. Cells contain a mixture of wild-type and mutant mtDNA molecules in heteroplasmic disorders, and disease expression only occurs when the mutant mtDNA load exceeds a threshold. Stochastic segregation of mtDNA molecules during development can therefore cause variable tissue expression of disease.

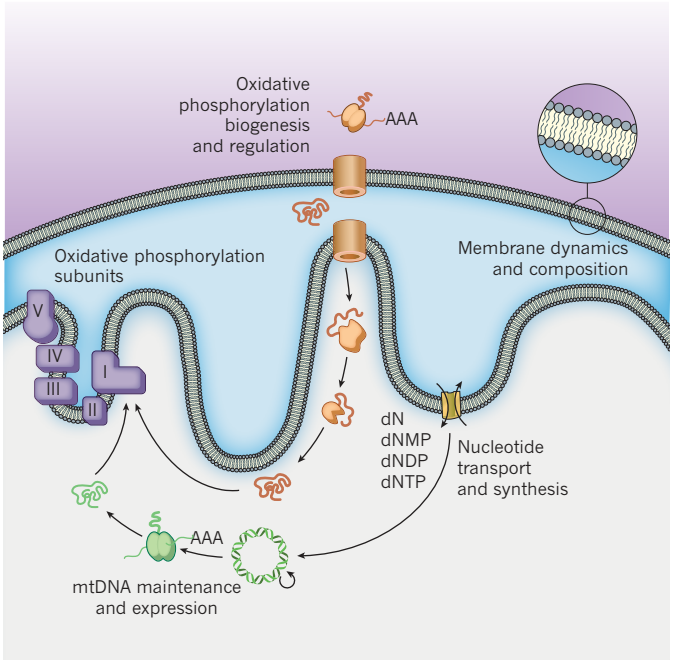

Figure 4 | Genetic pathways underlying mitochondrial respiratory chain disorders. The genes that are known to be mutated in respiratory chain disorders can be grouped in five broad categories on the basis of the pathway in which they participate. The products of genes identified so far are listed in Table 1, and include those involved in disorders of individual oxidative phosphorylation subunits of complexes I–V; proteins involved in mtDNA replication, transcription or translation; proteins involved in the assembly of oxidative phosphorylation complexes, mitochondrial protein import and protein homeostasis (grouped under the pathway of oxidative phosphorylation biogenesis and regulation); the proteins that are involved in nucleotide transport and synthesis; and proteins that are involved in the control of membrane composition and dynamics. The pathways in this figure are adapted from refs 47 and 94.

Mutations in nuclear genes

In 1995, the first underlying nuclear gene mutation of a mitochondrial disorder was identified when the Munnich and Rötig groups reported mutations in *SDHA* in a patient with complex II deficiency and Leigh syndrome[46]. Sequencing of the human genome, combined with characterization of the mitochondrial proteome[47], has propelled the discovery of nuclear disease genes and pathways that are required for respiratory chain assembly and function. Based on recent compilations[47,48] and disease databases (http://omim.org), there are now over 110 nuclear genes that are known to be mutated in respiratory chain disease, and this number is rapidly expanding. The genes that have been identified so far can be organized into five broad pathways involved in the expression, assembly and activity of the oxidative phosphorylation system (Fig. 4 and Table 1).

Mutations in the nuclear-encoded tRNA synthetases underscore the phenotypic heterogeneity that can be associated with the same pathway. Although all of these gene products facilitate translation of 13 mtDNA-encoded respiratory chain proteins, their clinical presentations are quite distinct[48]. For example, mutations in *EARS2* present as leukoencephalopathy and high cerebrospinal fluid lactate, *YARS2* as myopathy and sideroblastic anaemia, *HARS2* as ovarian failure, *AARS2* as hypertrophic cardiomyopathy and *SARS2* as pulmonary hypertension and renal failure. Moreover, these phenotypes do not match those arising from mutations in the corresponding tRNAs that are encoded by the mitochondrial genome. Mitochondrial heterogeneity — perhaps at the level of the nuclear-encoded mitochondrial ribosome[7] — or moonlighting roles of tRNA synthetases[49] may underlie the tissue-specific pathology.

Although most of the nuclear genes that underlie respiratory chain disease encode mitochondrial proteins, the small subset that do not provides valuable insight into the cross-talk between the organelle and the rest of the cell. For example, as already mentioned, mitochondria are reliant on some cytosolic pathways for proper nucleotide homeostasis, and mutations in the genes that encode ribonucleotide reductase, *RRM2B* (ref. 12), and thymidine phosphorylase, *TYMP* (ref. 50), disrupt mtDNA maintenance. Mutations in *WFS1*, which encodes an endoplasmic-reticulum resident protein, cause Wolfram syndrome that is characterized by deafness, diabetes mellitus, diabetes insipidus and optic atrophy. Although not formally classified as a mitochondrial disorder, the phenotypic overlap and presence of mtDNA deletions in some patients has led to speculation that WFS1 mediates interactions between the endoplasmic reticulum and mitochondria[51]. This hypothesis is supported by the observation in *Saccharomyces cerevisiae* that a component of the endoplasmic-reticulum–mitochondria encounter structure[52], MMM1, co-localizes with mtDNA nucleoids and has a role in mtDNA stability[53]. We anticipate that research into the genetics of mitochondrial disorders will continue to reveal unexpected connections, either physical or functional, between mitochondria and the rest of the cell.

Environmental modifiers

Environmental factors can influence the course of genetic mitochondrial disorders, and even phenocopy them. For example, tobacco use and heavy consumption of alcohol are risk factors for loss of vision in LHON[54]. Exposure to toxic substances can produce pathology that resembles LHON, as was seen in an epidemic of blindness that occurred in Cuba in the early 1990s. The cause was ultimately

Table 1 | Products of genes that are known to be mutated in respiratory chain disorders grouped by pathway

Oxidative phosphorylation subunits	mtDNA maintenance and expression	Oxidative phosphorylation biogenesis and regulation	Nucleotide transport and synthesis	Membrane dynamics and composition
Nuclear encoded				
Complex I: NDUFA1, NDUFA2, NDUFA9, NDUFA10, NDUFA11, NDUFA12, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2 **Complex II**: SDHA, SDHB, SDHC, SDHD **Complex III**: UQCRB, UQCRQ **Complex IV**: COX4I2, COX6B1 **Complex V**: ATP5E	TWINKLE, MTFMT, GFM1, LRPPRC, MPV17, MRPS16, MRPS22, POLG, POLG2, TRMU, TSFM, TUFM, C12orf65, MTPAP, MRPL3, SARS2, YARS2, HARS2, MARS2, AARS2, RARS2, EARS2, DARS2, TACO1, MTO1, RMND1, PNPT1, PUS1	**Complex I**: NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, ACAD9, FOXRED1, NUBPL **Complex II**: SDHAF1, SDHAF2 **Complex III**: BCS1L, HCCS, TTC19 **Complex IV**: COX10, COX15, ETHE1, FASTKD2, SCO1, SCO2, SURF1, COX14, COA5 **Complex V**: ATPAF2, TMEM70 **Fe–S**: ABCB7, FXN, ISCU, NFU1, BOLA3, GLRX5 **Other**: DNAJC19, GFER, HSPD1, SPG7, TIMM8A, AIFM1, AFG3L2	DGUOK, RRM2B, SLC25A3, ANT1, SUCLA2, SUCLG1, TK2, TYMP	ADCK3, AGK, COQ2, COQ6, COQ9, DRP1, MFN2, OPA1, PDSS1, PDSS2, TAZ, SERAC1
mtDNA encoded				
Complex I: ND1, ND2, ND3, ND4, ND4L, ND5, ND6 **Complex III**: CYTB **Complex IV**: COX1, COX2 **Complex V**: ATP6, ATP8	12S rRNA, tRNATyr, tRNATrp, tRNAVal, tRNAThr, tRNASer1, tRNASer2, tRNAArg, tRNAGln, tRNAPro, tRNAAsn, tRNAMet, tRNALeu1, tRNALeu2, tRNALys, tRNAIle, tRNAHis, tRNAGly, tRNAPhe, tRNAGlu, tRNAAsp, tRNACys, tRNAAla			

List of gene products was generated through synthesis of existing compilations of genes known to be mutated in respiratory-chain disease[47,48], as well as review of the literature.

found to be widespread folate deficiency combined with methanol toxicity from homemade rum[55]. Formate, a by-product of methanol metabolism, accumulates in the setting of folate deficiency and causes inhibition of complex IV.

Certain medications have long been known to have toxic effects on mitochondrial function. Owing to the bacterial origins of the mitochondrial ribosome, mtDNA translation can be adversely affected by antibiotics such as aminoglycosides, which can cause sensorineural deafness when administered at high doses. Individuals with certain mtDNA mutations in the 12S ribosomal rRNA — estimated to have a population prevalence of 0.19% — are predisposed to deafness from aminoglycosides, and can experience hearing loss even with short exposure to the recommended doses[56,57]. The viral origins of the mitochondrial DNA polymerase make it susceptible to nucleoside analogue antivirals (including fialuridine). This class of drugs is used commonly for HIV, and can cause side effects such as lactic acidosis through mtDNA depletion[58].

Microorganisms are an emerging class of environmental modifiers, ranging from gut microbiota to viruses. Gut bacteria are drivers of disease progression in ethylmalonic encephalopathy. The causal gene, *ETHE1*, encodes an enzyme that detoxifies sulphur compounds, which are released by gut microbiota, and its loss results in H_2S accumulation, with subsequent inhibition of complex IV and short-chain acyl-CoA dehydrogenase. Treatment with metronidazole (an antibiotic that reduces gut microbial content) and *N*-acetylcysteine (which promotes glutathione-mediated detoxification of H_2S) results in clinical improvement[59]. Although a role for viral infections in the course of mitochondrial disorders has not been identified, experimental studies indicate that some viruses, such as HIV[60], can modulate complex I activity.

Unsolved cases of mitochondrial disease

Over the past 25 years, most of the mitochondrial disease genes have been identified in familial forms of disease, in which it is possible to follow the segregation of highly penetrant causal alleles. In our experience at Massachusetts General Hospital, we have found that less than 25% of patients with clinical and biochemical evidence of mitochondrial disease have strong evidence of an affected first- or second-degree relative. Several recent studies have applied exome sequencing to establish molecular diagnoses in singleton cases[61,62]. However, the success rate is lower than anticipated. Our experience has shown that in biochemically proven, severe cases in infants exome sequencing should achieve a diagnosis in about half of cases[62]; however, the success rate is projected to be much lower in milder cases of disease, or those with adult onset.

How can we explain these unsolved cases? Most exome studies of singleton cases have been powered to identify recessive mutations in mitochondrial proteins. It is possible that these unsolved cases are a result of dominant-acting, subtle recessive or regulatory mutations with incomplete penetrance, all of which are difficult to identify over the background of polymorphisms. Alternatively, these cases could be due to mutations in regions of DNA that are not targeted for sequencing. Some cases could also have purely environmental causes, as with the Cuban blindness epidemic. A tantalizing possibility is that a subset of these unsolved cases is due to complex genetic inheritance, as a result of interactions between gene variants that each have weak or synergistic effects, known as synergistic heterozygosity[63]. Targeted exome sequencing studies have reported that healthy controls will typically carry a burden of about 15–20 heterozygous, loss-of-function protein alleles within their mitochondrial proteomes[62]. This high burden of deleterious alleles is probably tolerated because of the robustness of mitochondrial networks. However, it is possible that mutations that affect multiple genes, operating in the same or parallel pathways, may conspire to yield pathology. Notably, there is suggestive evidence that supports synergistic interactions between mtDNA and nuclear DNA variants[64]. Defining the genetic architecture of the large number of unsolved sporadic cases of mitochondrial disease represents the next major challenge of mitochondrial genetics.

Mitochondrial ripples and responses

Despite remarkable progress in defining the genes and environmental triggers that underlie mitochondrial disease, their pathogenesis remains almost a complete enigma. Many medical textbooks offer the oversimplified explanation that disease manifests in tissues with the highest ATP demand, or because of oxidative damage. Although these factors probably contribute to disease, the picture is much more complicated. Cellular models of disease indicate that there is a remarkable capacity for preservation of ATP production through enhanced glycolysis[65], and, in animal models of respiratory chain dysfunction, pathology can develop without a major increase in oxidative damage[66,67]. Furthermore, the fact that inhibition of respiration can be tolerated, and even beneficial in certain settings (Box 1), indicates that the consequences of respiratory chain lesions are not uniformly bad, and suggests the involvement of nonlinear modes of pathogenesis and threshold effects.

BOX 1

Can inhibition of the respiratory chain be beneficial?

Although respiratory chain inhibition is often viewed as undesirable, several observations have suggested that the consequences can be beneficial in certain situations. Metformin, the most commonly prescribed oral medication for type 2 diabetes mellitus, inhibits complex I. This bioenergetic effect is thought to contribute to metformin's inhibition of hepatic gluconeogenesis[95]. Lactic acidosis is a known — although infrequent — side effect of metformin treatment, highlighting the fine balance between therapeutic and toxic effects of respiratory chain inhibition. Pretreatment with small-molecule inhibitors of the respiratory chain can protect organs, such as the brain and heart, against ischaemia–reperfusion injury[96]. Finally, data from studies in both the roundworm *Caenorhabditis elegans*[97] and the fruitfly *Drosophila melanogaster*[98] indicate that RNA-interference-mediated knockdown of several components of the respiratory chain extends lifespan. The general theme that emerges from all of these observations is that the cellular and organismal consequences of modest respiratory chain inhibition can be used for therapeutic purposes — a concept that some have termed mitochondrial hormesis. We suspect that therapeutic effects occur when the balance of responses to a respiratory chain lesion weighs in favour of homeostasis as opposed to pathogenesis.

To systematically understand pathogenesis, it is convenient to consider the proximal consequences of respiratory chain lesions — or mitochondrial ripples — and the secondary cellular responses they evoke. Model-organism studies provide several examples of such ripple–response cascades. The 'retrograde' response in *S. cerevisiae* is a defined transcriptional response that allows the survival of that organism in the setting of an impaired respiratory chain, mainly by compensating for incomplete TCA-cycle function to maintain production of metabolites such as glutamate[68]. The precise ripples that trigger the yeast retrograde response remain unclear, although loss of the PMF is thought to have a key role[69]. In the fruitfly *Drosophila melanogaster,* respiratory chain impairment can trigger blockade of the G1–S transition of the cell cycle through two possible ripple–response pathways: mutation of a complex-I subunit leads to ROS-mediated activation of JNK signalling[70], whereas diminished ATP production as a consequence of a complex-IV mutation causes AMPK activation[71]. Studies in the roundworm *Caenorhabditis elegans* have defined a mitochondrial unfolded protein response (UPR^{mt}) that is activated by disturbed protein homeostasis resulting from insults to the respiratory chain[72]. The UPR^{mt} comprises increased expression of mitochondrial chaperones, and, notably, mutations in this pathway can give rise to several neurodegenerative disorders[73]. The lessons that have emerged from model-organism studies are that a broad range of ripple–response pairs can result from respiratory chain lesions, and that these pairs confer remarkable tolerance to such insults. However, some of these may be adaptive on short timescales and pathology-inducing over longer timescales.

Metabolomics studies have attempted to systemically catalogue biochemical ripples that emanate from respiratory chain inhibition. One study characterized the effect of small-molecule inhibitors of the respiratory chain on metabolite flux in cultured cells[74] and correlated these changes to patient plasma measurements. Specific blockade of complex III resulted in decreased production of uridine, which was consistent with the long-standing observation that respiratory chain-deficient cells are uridine auxotrophs[75]. Lactate secretion highlighted that the induction of the lactate dehydrogenase reaction is a means to support glycolytic ATP production and consume NADH to maintain redox cofactor balance. Another study reported that reverse flux through the TCA cycle, specifically reductive carboxylation of α-ketoglutarate to isocitrate, is a response to respiratory chain inhibition[76].

Ideally, investigation of ripple–response cascades should explain the end pathology. One promising cascade involves calcium. Mitochondria have a major role in shaping the cytosolic calcium concentrations through uptake with a uniporter[77–79], which is dependent on an intact PMF. Several studies have converged on the mechanism that an increase in cytosolic calcium, secondary to loss of mitochondrial PMF, is a key signalling intermediate in response to respiratory chain lesions through activation of calcium-sensitive signalling factors (such as calcineurin and calcium/calmodulin-dependent protein kinase IV (CaMKIV)[80,81]). We hypothesize that activation of calcium-dependent signalling may in fact help to explain several pathological hallmarks (Fig. 1). For example, activation of CaMKIV can induce mitochondrial biogenesis[82], potentially contributing to the finding of 'ragged red fibres' that are often seen on skeletal-muscle biopsy. Activation of calcineurin has also been found to induce hypertrophic cardiomyopathy[83]. Altered calcium dynamics in gastrointestinal interstitial cells of Cajal compromise their pacemaking activity[84], and this may contribute to intestinal pseudo-obstruction (Fig. 1b). However, not all respiratory chain mutations disrupt calcium homeostasis[85]. Moreover, mitochondrial calcium buffering can vary across tissues[86], and this may shape the pattern of tissue expression.

Ripple–response cascades can flow outside of cells, leading to non-cell-autonomous effects. Lactic acidosis is the best known example, and can impair the function of multiple organs by reducing serum pH. Fibroblast growth factor 21, a hormone that mediates aspects of the starvation response, is released from the skeletal muscle of patients with mitochondrial myopathy[87]. This has led to the intriguing hypothesis that it may drive systemic metabolic pathology. Recent evidence has pointed towards a crucial role for mitochondria in the regulation of innate immunity[88]; however, its role in mitochondrial disorders is largely unexplored.

Future prospects and challenges

Understanding the pathogenesis of mitochondrial disorders is an exciting new frontier, with many opportunities. First, as a group, these disorders affect at least 1 in 5,000 individuals, and there are no proven therapies[89]. There is, therefore, a special opportunity to develop therapeutics for patients who otherwise have distressingly few options. Second, these disorders are a continuous source of insight into basic cell biology, and much of what we know about respiratory chain assembly and compartmentalization of metabolism has come directly from studying them. Finally, there is great interest in the role of mitochondria in many common human diseases, which is fuelled by the observation that many common, degenerative disorders are associated with a quantitative decline in mitochondrial activity. Distinguishing cause from correlation, however, is challenging and mitochondrial disorders may be valuable tools for clarifying the organelle's role in common disease. Capitalizing on these opportunities will require that we fully decipher the pathogenesis of these orphan diseases.

We anticipate that two complementary lines of investigation, cell- and patient-based, will be required to meet this challenge. First, it will be essential to characterize the network-level properties

of mitochondria in cell-based studies. As a result of classic biochemical studies and (more recent) proteomic studies of isolated mitochondria, we have a reasonable knowledge of the organelle's protein inventory and reaction repertoire, as well as a theoretical framework for understanding regulatory control. Moving forward, it will be crucial to experimentally map interactions among all of its components, both at a physical and genetic level, across different cell types. Initial progress has already been made in this area, with the recent production of a genetic interaction map, focusing on *S. cerevisiae* mitochondria[90]. The organelle's rich evolutionary history and transcriptional regulation will facilitate computational identification of modules of functionally interacting genes[91]. These network-level maps will reveal the homeostatic mechanisms that buffer against environmental or genetic insults. Second, in parallel, it will be crucial to catalogue genotypes and phenotypes from individual patients throughout the world. Next-generation sequencing is already facilitating sequencing of patient genomes, while Internet- and wireless-enabled technologies will yield in-depth phenotypes with unprecedented temporal resolution. Because the individual mitochondrial disorders are so rare and diverse, we anticipate that an open-source, collaborative model — in which patients and their doctors are partners for biomedical research — will be required so that data can be aggregated and shared for discovery. Metabolic profiles, which are obtained from perturbed cells grown in culture as well as from human plasma[74], may represent the key ingredient for connecting cell-level and patient-level data into predictive models of pathogenesis.

Identifying a link between genotype and phenotype is a challenge for all diseases. But features unique to mitochondria — their relatively well-studied biochemistry, a near-complete protein parts list, an ability to study mitochondria in isolation or *in situ*, the large number of monogenic disorders, and an active and engaged patient community — provide key advantages that promise to place this organelle at the forefront of the burgeoning field of medical systems biology.

Studies of orphan mitochondrial disorders will be pivotal in solving one of the most important problems in fundamental metabolism: the logic of compartmentalization. Why have certain pathways persisted within mitochondria, whereas others have been duplicated or fully relocated to the cytosol? Mitochondria have retained a bacterial type-II fatty-acid synthesis pathway, as well as duplicate versions of tetrahydrofolate-dependent one-carbon metabolism and the gluconeogenic enzyme phosphoenolpyruvate carboxykinase. What advantage is conferred by the gain or loss of such pathways? We anticipate that studies of the rare mitochondrial disorders will shed light on the *in vivo* relevance of these pathways, and the circumstances in which they are operative. New ways of measuring compartment-specific metabolism, both in cultured cells and *in vivo*, will be required to drive this field forward.

We can be optimistic that understanding mitochondrial pathogenesis will enable the development of new therapeutics for these devastating disorders, some of which may also be useful for the treatment of common diseases. An example of such repurposing can be seen in the history of dichloroacetate, a small molecule that reduces lactic acid production by stimulating pyruvate dehydrogenase. Dichloroacetate was tested for the treatment of lactic acidosis in mitochondrial disorders but lacked clinical efficacy and resulted in toxic side effects[92]. Although it was largely unsuccessful for use in the treatment of mitochondrial disorders, it has since been repurposed and is now being tested in clinical trials for some cancers. Many cancers have an increased reliance on aerobic glycolysis with concomitant lactate production — the so-called Warburg effect. Dichloroacetate is being used to reverse this metabolic hallmark, with promising early results in human trials[93]. We anticipate that this pattern will continue, and that drug development for mitochondrial disorders will ultimately prove beneficial for patients with these rare disorders, as well as those with more common disease. ■

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