

Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction

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Anna S. Monzel¹, José Antonio Enríquez^{2,3} & Martin Picard^{1,4,5}✉

Mitochondria have cell-type specific phenotypes, perform dozens of interconnected functions and undergo dynamic and often reversible physiological recalibrations. Given their multifunctional and malleable nature, the frequently used terms ‘mitochondrial function’ and ‘mitochondrial dysfunction’ are misleading misnomers that fail to capture the complexity of mitochondrial biology. To increase the conceptual and experimental specificity in mitochondrial science, we propose a terminology system that distinguishes between (1) cell-dependent properties, (2) molecular features, (3) activities, (4) functions and (5) behaviours. A hierarchical terminology system that accurately captures the multifaceted nature of mitochondria will achieve three important outcomes. It will convey a more holistic picture of mitochondria as we teach the next generations of mitochondrial biologists, maximize progress in the rapidly expanding field of mitochondrial science, and also facilitate synergy with other disciplines. Improving specificity in the language around mitochondrial science is a step towards refining our understanding of the mechanisms by which this unique family of organelles contributes to cellular and organismal health.

To guide scientific progress, medicine has developed a detailed cartography of the multiple functions accomplished by unique body parts and organ systems. The resulting classification system of physiological functions displayed in every medical textbook is the foundation of modern medicine¹ (Fig. 1a). Likewise, cell biology recognizes dozens of distinct cellular functions and behaviours that interact to sustain organismal health (Fig. 1b). This detailed cartography of cellular functions and behaviours has guided our theories around how cells divide and differentiate, communicate and interact with one another, has led to discoveries of disease mechanisms and has motivated the development of laboratory methods and specialized instruments to capture the multifaceted nature of different types of living cells. Specificity begets biological insights.

As for organs and cells, several productive decades of mitochondrial research have shown that mitochondria are similarly multifaceted (Fig. 1c). However, despite considerable advances in establishing the molecular machinery and functional diversity among mitochondria, we lack a systematic nomenclature to reflect the full spectrum of functional, morphological and molecular domains of mitochondrial biology. As mitochondria have become the most studied organelle across the biomedical sciences², establishing a logical framework to teach and study the multifaceted nature of mitochondria becomes paramount. In this Perspective, we examine how infusing further specificity in our classification system for mitochondrial science can propel the field towards increasingly meaningful insights into human health.

¹Department of Psychiatry, Division of Behavioral Medicine, Columbia University Irving Medical Center, New York, NY, USA. ²Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain. ³CIBER de Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain. ⁴Department of Neurology, H. Houston Merritt Center, Columbia Translational Neuroscience Initiative, Columbia University Irving Medical Center, New York, NY, USA. ⁵New York State Psychiatric Institute, New York, NY, USA. ✉e-mail: martin.picard@columbia.edu

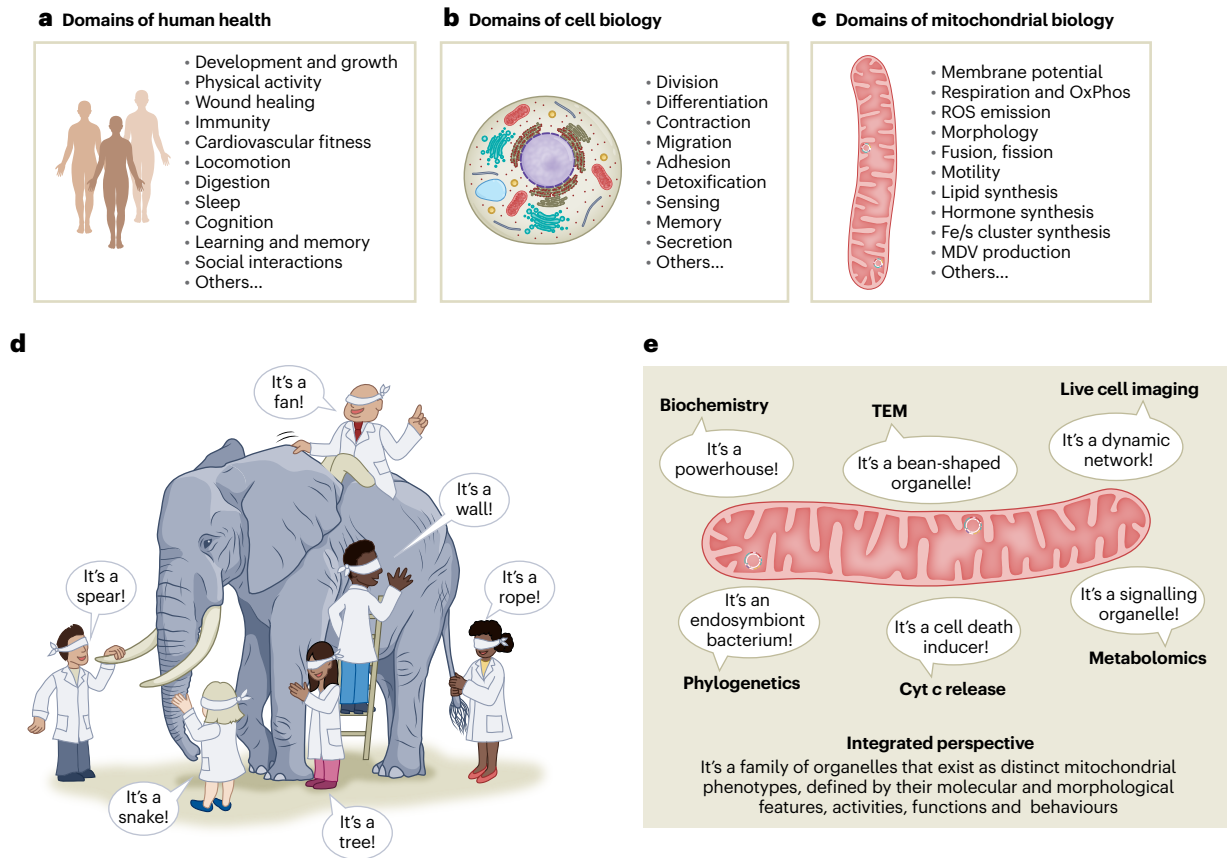


Fig. 1 | An integrative approach to mitochondrial biology. **a–c**, There are organ-specific domains of human health that guide medical investigation and practice (**a**), cell-type-specific domains of cell biology that guide biological theories and research practices (**b**) and domains of mitochondrial biology that guide mitochondrial science (**c**). **d**, Blind men—here scientists blindfolded by their perspective limited by prevailing theories, training and career history, available instrumentation and/or analytical methods—make valid observations about specific aspects of the animal under study. However, without a global perspective enabling them to perceive the whole animal at once, an error of logic is committed, leading to erroneous induction and conclusions, drawn from its parts, about the nature of the whole organism. **e**, The same type of limitation

exists when examining mitochondria with different instruments that are blind to other aspects of mitochondrial biology, for example, microscopy, biochemistry, genomics and metabolomics. Focused reductionist approaches are necessarily undertaken blind to other domains of function or behaviour, yielding partially valid conclusions and, when taken in isolation, a largely inaccurate picture of the system/organelle. The history of mitochondrial research illustrates the need for interdisciplinary approaches and sufficiently specific terminology to examine mitochondrial biology and its contribution to human health. ROS, reactive oxygen species; MDVs, mitochondria-derived vesicles; TEM, transmission electron microscopy; Cyt c, cytochrome c.

The need for specificity in mitochondrial science

The powerful ‘powerhouse of the cell’ analogy³ does not capture the multifunctional and malleable nature of these fantastic organelles; the analogy is too simplistic and incomplete (Fig. 1d,e). Overly simplistic analogies risk impeding progress in mitochondrial science in two main ways. First, incomplete analogies like the ‘powerhouse’ create a monofunctional image of these complex organelles. This narrow mental image limits the spectrum of possible mechanisms by which mitochondria may contribute to the organism’s biology and physiology. In the same way that all cells are recognizable by shared gross anatomical features (for example, cell membrane and nucleus), mitochondria share a recognizable double membrane structure, cristae and a circular genome. Yet at a higher level of resolution, cell subtypes and their mitochondrial subtypes both qualitatively and quantitatively differ in their specific molecular features, activities, functions and behaviours. Unlike powerhouses whose sole function is energy transformation, mitochondria are multifaceted and multifunctional.

The second way in which outdated analogies can impede the field’s progress is by shaping terminology⁴. For established investigators

with decades of historical perspective, some concepts may transcend terminology. However, to newcomers in the field, terminology is the basis of understanding. A powerhouse transforms energy, and the only way in which powerhouses can be ‘dysfunctional’ is by exhibiting diminished energy production capacity. But mitochondria are multifunctional, performing dozens of different functions (see below). As a result, they can malfunction in multiple different ways. Mitochondria also are malleable, capable of dynamically and reversibly adapting to energetic, environmental and other stressors^{5–7}. Temporary increases or decreases among molecular features and functions are the basis of adaptation—not necessarily signs of dysfunctional processes. Thus, letting go and clearing the mind of the simplistic analogy challenges the concept of ‘dysfunction’ and allows investigators to intuitively interpret dynamic changes more physiologically, and therefore more accurately.

Studying ‘human function’ or ‘cellular function’ would strikingly lack specificity and meaning for medicine and cell biology. Similarly, ‘mitochondrial function’ is devoid of the specificity required to advance our science. For the two reasons noted above, we are forced to conclude that the terms ‘mitochondrial function’ and ‘mitochondrial

dysfunction' are flawed, misleading misnomers, and should generally be avoided. To formulate accurate models and hypotheses about the mechanisms in which mitochondrial biology contributes to complex cellular and organismal processes, mitochondrial science needs more specific terminology.

In this Perspective, we first provide quantitative examples of the diversity of mitochondrial phenotypes, highlighting how mitochondria in different organs and cell types molecularly, functionally and morphologically specialize. We then propose a general conceptual framework and hierarchical terminology system to help guide the evolving field of mitochondrial science. Our collective goal should be to develop a logical framework that describes the many ways in which mitochondria dynamically adapt to perturbations, become mismatched to their metabolic environment or acquire specific defects that manifest into one or more quantifiable aspects of mitochondrial biology. The proposed framework can help guide both our theories and measurement approaches to capture the rich diversity of mitochondrial functions, and thus elucidate how they shape health or contribute to disease states.

Mitochondria are multifunctional and essential for life

As a family of organelles, mitochondria perform several functions beyond oxidative phosphorylation (OxPhos) that are essential to multicellular life. Table 1 provides a list of known mitochondrial functions and mitochondrial behaviours. In the same way that all cell types share some fundamental functions (for example, gene expression, protein synthesis and signal transduction), all mitochondria harbour the core OxPhos system. The OxPhos system is a set of five multi-protein complexes, four of which—known as the electron transport chain (ETC)—funnel free electrons from reducing equivalents to molecular oxygen, harnessing in the process the resulting free energy (ΔG) to build an electrochemical gradient ($\Delta\Psi_m + \Delta pH$) across the inner mitochondrial membrane (IMM). Electrons are also being injected into the ETC through non-canonical sources including the glycerol phosphate shuttle⁸ and other enzymes in specific cell types⁹. The resulting transmembrane potential is the driving force for several mitochondrial functions, including the uptake of ions (Ca^{2+} , Na^+ , Mn^{2+} and others)¹⁰, import of nuclear-encoded proteins and pre-proteins¹¹, synthesis of iron–sulfur (Fe/S) clusters^{12–14}, antioxidant defences regenerated by nicotinamide nucleotide transhydrogenase (encoded by *NNT*)¹⁵, antiviral signalling^{16–19}, ATP synthesis by the F_0F_1 ATP synthase (complex V)²⁰, among many others. Membrane potential is also required to maintain the integrity of the mitochondrial permeability transition pore²¹, to regulate crista shape and for mitochondrial fusion/fission dynamics²².

In experimental settings, cells can survive without OxPhos, but cannot survive without energized mitochondria. The fundamental function that requires mitochondria but not OxPhos is Fe/S cluster synthesis²³. Fe/S clusters are essential to dozens of enzymatic reactions within the cell, and Fe/S cluster synthesis is in fact maintained in cultured cells whose mitochondria have lost their OxPhos capability²⁴.

Biosynthetic mitochondria

Beyond energy production and Fe/S cluster biosynthesis, mitochondria also perform anaplerosis²⁵. Tricarboxylic acid (TCA) cycle flux connects cataplerosis and anaplerosis²⁵ and release of metabolites (for example, citrate) used in the cytoplasm for the synthesis of lipids and other macromolecules²⁶. Like cells that exhibit metabolic substrate preferences (for example, neurons do not oxidize lipids, mainly lactate and ketone bodies), mitochondria catabolize various substrates (for example, pyruvate, glutamine, fatty acids and other amino acids) with different affinities²⁷. The biosynthetic capacity of some specialized mitochondria contributes to producing molecules required for cell growth and division (for example, nucleotides, amino acid and haem)²⁷, and also to synthesizing endocrine signalling molecules released systemically².

Signalling mitochondria

Mitochondria are systemic signalling hubs². They contribute to transducing information both within and between cells^{28,29}. Inside the cell, they release metabolites for signalling in the nucleus where mitochondria-derived molecules are the required substrates and cofactors of epigenetic modifications on the DNA and histones^{30,31}. Succinate for instance is an obligatory metabolic intermediate produced in the mitochondrial matrix, which acts both intracellularly and as an intercellular messenger³². Some mitochondria also specialize in producing systemic signals. Mitochondria in the gonads and adrenal cortex specialize in steroidogenesis, the production of broad-acting messengers including the sex-defining hormones (oestrogens, progesterone and testosterone), and the glucocorticoids and mineralocorticoids that ensure preparedness to metabolic and psychological stressors^{33,34}. In turn, some mitochondria sense extracellular paracrine and endocrine inputs, including succinate, steroid hormones and other molecular signals using DNA-binding receptors and G-protein-coupled receptors on their surface^{35–38} (reviewed in ref. 2). Organisms are complex communication networks composed of specialized mitochondrial phenotypes.

Mitochondrial signalling also takes other more complete forms involving intercellular mitochondrial transfer. Molecular mitochondrial components, including the mitochondrial DNA (mtDNA), can be incorporated in extracellular vesicles and exosomes³⁹, released as cell-free mtDNA⁴⁰ and induce responses in target/recipient cells³⁹. Moreover, whole functional mitochondria are exchanged between adjacent cells during normal development⁴¹, in response to stress between tissues^{42,43} and between brain cell types^{44,45}, and as part of normal tissue maintenance in the heart⁴⁶. Whole mitochondria are also found at high levels in the human circulation⁴⁷, although their origin and destination(s) remain unclear. The extent of the intercellular cross-talk between mitochondria, and the influence of these processes on the maintenance of health largely remain to be determined.

Mapping mitochondrial diversity across tissues and cell types

Discovering mitochondrial diversity

Mitochondria are remarkably pleiotropic. The notion that mitochondria specialize into distinct tissue-specific mitochondrial phenotypes was first illustrated in two main ways: by imaging mitochondria with different Ca^{2+} dynamics across two main cytoplasmic compartments in cultured pancreatic acinar cells⁴⁸, and by quantifying proteomic differences among isolated mitochondria from rat brain, heart, liver and kidney^{49,50}. Subsequent expansion of this latter effort led to MitoCarta, a curated compendium of mitochondrial proteins in mice that revealed both quantitative and qualitative tissue-based mitochondrial differences⁵¹. This dataset, now in its third iteration as MitoCarta3.0 (ref. 52) and other high-confidence databases of mitochondrial proteins (for example, MitoCoP⁵³), have provided rich resources for studying over a hundred biochemical pathways and discovering dozens of new mitochondrial proteins.

In parallel with the elucidation of the mitochondrial proteome, the field has discovered substantial intracellular morphological and molecular heterogeneity of both genetic and non-genetic origin across different cell types and tissues^{54–59}. Mitochondria from the mouse brown adipose tissue, heart, liver and kidney exhibit large variation in the expression and biochemical activity of different OxPhos components and matrix dehydrogenases (which, in turn, shape their differential ability to oxidize different carbon substrates)^{60,61}. Together with our understanding of the divergent bioenergetic requirements and physiological functions of different organ systems⁶², a systematic understanding of mitochondrial specialization—at the molecular, functional and morphological levels—provides a basis to specify how mitochondrial phenotypes in different organs, cell types and subcellular compartments fulfil specific cellular and organismal functions².

Table 1 | Mitochondrial functions and behaviours

	Description	Reviewed in ref(s).	Methods described in ref(s).
Functions			
Membrane potential generation	Formation of the electrochemical gradient ($\Delta\Psi_m + \Delta pH$) across the IMM, usually by the electron pumping capacity of the respiratory complexes I, III and IV, but also by other processes including through ATP hydrolysis by the F_0F_1 ATP synthase (complex V).	104	105,106
Amino acid metabolism	Lysine metabolism (lysine- α -ketoglutarate reductase, encoded by AASS). Electrogenic malate-aspartate shuttle system, which is important for balancing pyridine dinucleotide redox states across subcellular compartments. Branched-chain keto and amino acids. Choline and derivatives as structural precursors for lipoproteins, membrane lipids and the neurotransmitter acetylcholine. Betaine as osmoregulator and an intermediate in the cytosolic transsulfuration pathway.	107–111	112–119
Ascorbate metabolism	L-ascorbate (vitamin C) biosynthesis in many plants and animals, but not in primates, which serves as osmoregulator and antioxidant. Mitochondria may recycle oxidized (dehydro)ascorbic acid.	120	121,122
Bicarbonate metabolism	Production of bicarbonate (HCO_3^-) by mitochondrial carbonic anhydrase V (encoded by CA5A), used as a cofactor for anaplerotic reactions (for example, ureagenesis and gluconeogenesis) and acid–base balance. The TCA cycle is an important contributor to cellular/extracellular acidification due to CO_2 production.	123	–
Calcium uptake and extrusion	Uptake of cytoplasmic Ca^{2+} via the mitochondrial calcium uniporter in a $\Delta\Psi_m$ -dependent manner; extrusion by the sodium/calcium exchanger NCLX (encoded by SLC8B1).	124–126	127,128
Hydrogen sulfide detoxification	Mitochondrial sulfide quinone oxidoreductase (encoded by SQOR) oxidizes hydrogen sulfide to glutathione persulfide by reducing CoQ.	129–132	133
Heat production	Heat generation is stimulated by uncoupling $\Delta\Psi_m + \Delta pH$ from ATP synthesis (thereby increasing electron flux and respiration) by UCP1 (encoded by UCP1), the ADP/ATP carrier (AAC, also ANT1), or by creatine-dependent substrate cycling and other futile cycles.	134–137	138
Intermediate metabolism	Enzymatic interconversion of metabolic intermediates to enable the synthesis of specific macromolecules, including five major anaplerotic ones. This includes the conversion of pyruvate into oxaloacetate by pyruvate carboxylase (encoded by PC), a critical step for de novo glucose synthesis (gluconeogenesis); citrate export to the cytoplasm where it is used for lipid synthesis or converted to acetyl-CoA for acetylation reactions; synthesis of itaconate, a derivative of cis-aconitate; succinate, α -ketoglutarate and others that participate in a variety of signalling process.	25,139,140	141,142
Fe/S cluster synthesis	Synthesis of Fe/S clusters, which serve as prosthetic groups of several essential proteins.	12–14	143
Light focusing	Mitochondria in the outer segment of the retinal photoreceptors acts as a ‘microlens’ that focuses incoming photons, increasing visual resolution.	144	–
Lipid oxidation	Beta-oxidation of long-chain, medium-chain and short-chain fatty acids into acetyl-CoA.	145	146
Lipid synthesis	Synthesis of cardiolipin and phosphatidylethanolamine from ER precursors in the IMM.	147–150	–
mtDNA maintenance and expression	mtDNA replication, transcription, protein synthesis and assembly of the OxPhos system.	151,152	153,154
Na⁺ import/export	Sodium (Na^+) uptake and release against cytoplasmic Ca^{2+} by the sodium/calcium exchanger protein NCLX (encoded by SLC8B1) or by Na^+/H^+ antiporter (molecular identity pending).	124,155	156
Neurotransmitter synthesis and degradation	Synthesis of the cofactor BH4 (tetrahydrobiopterin), used by hydrolase enzymes to synthesize catecholamines and neurotransmitters (serotonin, melatonin, norepinephrine and epinephrine) and nitric oxide. Mitochondria with OMM-anchored monoamine oxidases (encoded by MAOA and MAOB, donate electrons and contribute to electron flow in the ETC) also degrade catecholamines. Mitochondria also participate in GABA metabolism.	9,157	158,159
One-carbon metabolism and pyrimidine synthesis	The one-carbon metabolism connects the synthesis of nucleotides (purine and pyrimidine), amino acids (methionine, serine and glycine), S-adenosyl-methionine and folate. Ubiquinone-mediated oxidation of dihydroorotate to orotate by dihydroorotate dehydrogenase (encoded by DHODH) is a key step in pyrimidine synthesis.	160–163	164
OxPhos	Transduction of $\Delta\Psi_m + \Delta pH$ generated by the electron transport chain (ETC, also ‘respiratory chain’) into ATP synthesis by the F_0F_1 ATP synthase (complex V), abbreviated as OxPhos.	165	166

Table 1 (continued) | Mitochondrial functions and behaviours

	Description	Reviewed in ref(s).	Methods described in ref(s).
Oxygen sensing	The electron transport and free-radical generation by ETC complexes I and III is modulated by the partial pressure of oxygen, which can limit respiration at very low partial pressures of O ₂ .	167–170	–
Permeability transition	Opening of the high-conductance permeability transition pore (PTP), which dissipates membrane potential and promotes the release of intracristae and matrix-located components into the cytoplasm.	171,172	173–175
Protein import	Import, processing and folding of nuclear-encoded polypeptides from the cytoplasm by the translocator of the inner membrane (TIM) and outer membrane (TOM) complexes and associated proteins.	176	–
Redox homeostasis	Re-oxidation of enzymes and/or their redox cofactors (involved in anabolic and catabolic reactions) by the electron acceptors CoQ and cytochrome c (encoded by <i>CYTC</i>) within the mitochondrial respiratory chain, and production of NADPH by <i>NNT</i> .	177,178	–
Respiration	Electrons stored in reducing equivalents NADH and FADH ₂ , or derived from diverse redox reactions are sequentially delivered to respiratory complex I and CoQ, or cytochrome c, respectively, to promote the reduction of molecular oxygen at cytochrome c oxidase (complex IV).	179,180	181
ROS production	Production and release of ROS (H ₂ O ₂ , O ₂ ^{•−} , others) mainly at respiratory chain complexes I and III.	182,183	184
Steroidogenesis	Production of pregnanolone from cholesterol imported via IMM steroidogenic acute regulatory protein (encoded by <i>STAR</i>) followed by enzymatic transformation by P450 _{ssc} (encoded by <i>CYP11A1</i>) in the matrix. Intermediate or terminal steps for some steroids occur in the ER. Cytochrome P450 family members participate also in xenobiotic metabolism as well as bile acid and vitamin D biosynthesis.	33,34,185,186	187
Behaviours			
Antiviral signalling	Assembly of the mitochondrial antiviral signal (encoded by <i>MAVS</i>) adaptor protein on the OMM to potentiate downstream signalling, and activation of nuclear interferon pathways in the nucleus by mtDNA release.	39,188	–
Apoptotic signalling	Release of cytochrome c (encoded by <i>CYCS</i>), apoptosis-inducing factor (encoded by <i>AIF</i>), and other proteins that trigger different forms of cell death by acting on cytoplasmic and nuclear effectors.	189,190	–
Cristae remodelling	Dynamic remodelling of IMM cristae junctions, cristae shape and distribution via the combined action of optic atrophy 1 (encoded by <i>OPA1</i>) and mitochondrial contact site and cristae organizing system (MICOS) proteins.	103,191	95
DNA signalling	mtDNA extrusion in the cytoplasm, particularly in the form of oxidized mtDNA fragments via proteinaceous pores forming across the IMM and OMM, which trigger inflammasome activation.	189,190,192,193	175
Epigenetic remodelling	Transduction of mitochondrial states into changes in epigenome via several functions including metabolic intermediates, DNA release, ROS production and others.	30,194	–
Inter-organelle communication	Exchange of information between mitochondria and other organelles, particular the ER, where mitofusin 2 (encoded by <i>MFN2</i>) plays a key role in tethering organelles.	195,196	197,198
Mitochondrial dynamics	Mitochondrial fusion and fission through OMM-anchored and IMM-anchored GTPase proteins capable of merging or constricting mitochondrial membranes to enact fragmentation of larger organelles into smaller ones.	191,199–201	202
Mito–mito communication	Exchange of information between mitochondria by soluble signals (for example, ROS-induced ROS release, RIRR), by complete membrane fusion, or by physical extensions of thin protein-carrying OMM and IMM membrane protrusions (that is, nanotunnels) and trans-mitochondrial cristae alignment between energized mitochondria.	203–206	207–209
Motility	Movement of energized mitochondria across the cytoplasm via the combined action of motor and adaptor proteins interacting with cytoskeletal elements.	6,210	211
Vesicle formation	Release of MDVs destined to different cellular fates by the action of motor and accessory proteins acting on the OMM and IMM.	212	213,214

^aGeneration of mitochondrial membrane potential is the ‘mother’ of many other functions and behaviours, providing the driving force for the movement of ions, solutes and proteins across the IMM, the driving force for key enzymes and processes, including the phosphorylation of ADP into ATP (OxPhos). Mitochondrial features (that is, molecular components) and activities (individual enzyme and non-enzymatic activities) are too numerous to be comprehensively listed, so only functions and behaviours are included. CoQ, coenzyme Q.

Next, we provide examples where—as for cell types—mitochondrial phenotypes are quantitatively defined by their (1) functional characteristics, and (2) morphological and ultrastructural features.

Diversity in mitochondrial functions

Mitochondria differ between tissues, cell types and subcellular compartments. Here we provide four well-defined examples of mitochondrial functional specialization.

Blood leucocyte mitochondrial phenotypes. All leucocytes are derived from the same haematopoietic stem cells but differentiate in a metabolism-instructed manner⁶³ into distinct lineages, cell types and cell subsets that perform unique roles within the organism (Fig. 2a). Two main lineages exist: innate and adaptive immune cells. Within each lineage, say the adaptive lineage, both B and T lymphocytes are produced, and T cells differentiate into at least CD4⁺ helper and CD8⁺ cytotoxic cells. Each subtype also exists in their naïve state (not yet exposed to antigens), in an activated (effector) or in a memory state (holding the memory of antigen exposure).

Figure 2b shows the levels of citrate synthase (CS) activity (an imperfect proxy for mitochondrial content per cell), mitochondrial DNA copy number (mtDNAcn; the number of mtDNA copies per cell) and the enzymatic activity of OxPhos complexes within different immune cell types⁵⁹. Plotting these common mitochondrial features and activities on bivariate plots illustrates the different phenotypic space occupied by each cell subtype. In these plots, the diagonal (where both parameters scale in proportion to each other) reflects the same mitochondrial phenotype, and orthogonal deviations from the diagonal reflects distinct mitochondrial specialization/adaptation.

For example, natural killer cells and neutrophils have similarly low CS activity and mtDNAcn, compared to CD4⁺ and CD8⁺ T lymphocytes, which have similar CS activity but about two- to three-fold more mtDNA copies per cell. The naïve-to-memory transition may also lead to a convergence of mitochondrial phenotypes, as expected from their metabolic transitions. Plotting ratios of two features on both *x* and *y* axes also reveals distinct types of mitochondria—or mitotypes⁵⁹—among the two major immune cell lineages (Fig. 2c).

Skeletal muscle mitochondrial phenotypes. Two major classes of skeletal muscle cell types, or myofibres, coexist: oxidative or myosin heavy chain type I, and glycolytic or myosin heavy chain type II⁶⁴ (Fig. 2d). Like metabolically divergent immune cells that coexist within the circulation and lymphoid organs, oxidative and glycolytic muscle fibres coexist as a mosaic within the same skeletal muscle⁶⁵. Nevertheless, their mitochondria functionally differ from one another in three main ways. In rat skeletal muscles, compared to the glycolytic muscle (white gastrocnemius) mitochondrial phenotype, the oxidative muscle (soleus) phenotype (1) has a 50–90% lower affinity for ADP-driven respiration (requires more ADP to drive the same OxPhos flux) and depends heavily on creatine⁶⁵; (2) emits ~50% less hydrogen peroxide (H₂O₂)⁶⁵; and (3) has ~60% lower calcium retention capacity⁶⁶ (Fig. 2e–g). Thus, mitochondrial populations from closely related skeletal muscle cell types exhibit strikingly different multivariate mitochondrial phenotypes.

A related example concerns the further differentiation of skeletal muscle mitochondria into specialized subpopulations within the same cytoplasm. In each myofibre, two major mitochondrial subpopulations cohabit: subsarcolemmal (SS; also known as perivascular and perinuclear) and intermyofibrillar (IMF), which differ on a number of morpho-functional aspects⁶⁷. For example, compared to IMF, SS mitochondria have ~60% lower oxidative capacity⁶⁸, ~25–40% lower enzymatic activities for OxPhos enzymes⁶⁸, but ~60% higher cardiolipin content⁶⁸, and exhibit a greater capacity to oxidize lipids via beta-oxidation⁶⁹. Both subpopulations even undergo differential responses to exercise⁶⁹. As might be expected, these functional divergences between SS and IMF mitochondria are associated by distinct proteomes, notably highlighting the lower abundance of OxPhos proteins in SS compared to IMF mitochondria⁷⁰, accounting at least in part for their functional divergences.

Brown adipose tissue mitochondrial phenotypes. The last example is another instance of intracellular mitochondrial specialization in brown adipocytes where two mitochondrial phenotypes coexist in the same cytoplasm: the peridroplet mitochondria (PDM) surrounding

lipid droplets, and non-PDM (that is, cytoplasmic; Fig. 2h)⁷¹. Compared to the cytoplasmic type, the PDM have double the respiratory and ATP synthesis rates (pyruvate and malate oxidation), tend to have a higher $\Delta\psi_m$ and contain more of their OxPhos complexes assembled into supercomplexes (Fig. 2i)⁷². However, PDM have a lower oxidative capacity for fatty acids, are larger, less motile and fuse less often with surrounding mitochondria (Fig. 2j). Thus, functionally defined mitochondrial diversity extends across the cellular and intracellular levels⁵⁸.

Diversity in mitochondrial morphology

Functional specialization often comes with morphological specialization⁷³. Mitochondria undergo dynamic changes in morphology and function (that is, morphofunction⁷⁴), where changes in shape trigger downstream changes in functions^{75–77}. Changes in morphology occur within seconds to minutes, triggered by metabolic and endocrine signals^{78–82}. But distinct mitochondria in different cell types also develop relatively stable and sufficiently distinct ultrastructural characteristics (for example, cristae density and anatomy) that can allow a trained investigator to distinguish tissue-specific mitochondrial phenotypes by electron microscopy alone (Fig. 3a).

The gross morphology (tubular and branched versus rounded and fragmented) of mitochondria in different cell types, or within different subcellular compartments, can also be quite unique (Fig. 3b). For example, neuronal axonal mitochondria must travel long distances and tend to be smaller and shorter organelles, compared to much longer and branched dendritic mitochondria that are less mobile and contribute to local Ca²⁺ buffering, ATP production and possibly other roles^{83–85}. The neuron somata mitochondria (that is, cell body, where the bulk of mitochondrial biogenesis takes place, and which likely represent the source or ‘stem’ population feeding both axonal and dendritic mitochondrial pools) exhibit intermediate size and morphological complexity⁸⁵. In skeletal muscle, SS and IMF mitochondria within the same myofibres also are vastly recognizable by their morphologies alone in both mice and humans (Fig. 3c)^{67,86}, and they undergo specific morphological recalibrations to challenges such as exercise⁸⁷.

In general, as for the transition from stem cells to highly differentiated cellular arbours (for example, neurons), the acquisition of specialized cellular characteristics is associated with a transition from a relatively ‘undifferentiated’, rounded, depolarized and stem-like mitochondrial phenotype, towards specialized elongated and morphologically diverse mitochondrial phenotypes that perform tissue-specific functions and behaviours.

Taken together, the sections above illustrate how mitochondria diverge on at least three quantifiable levels: molecularly, functionally and morphologically. In the context of these rich and diverse tissue-type-specific and cell-type-specific mitochondrial phenotypes, mitochondrial science needs a terminology system that incorporates these notions to effectively guide scientific inquiry.

A new framework for mitochondrial terminology

Why we need more specific terminology

With the goal to best capture and incorporate critical aspects of mitochondrial biology in our daily discourse, we propose some standard terminology. This terminology system: (1) builds on the natural hierarchical organization of biological components from molecules (simple) to organelles (complex); and (2) draws from traditional classification systems around organ-based and cell-type-based terminology in the biomedical and biological sciences (Fig. 4).

Beyond function and dysfunction. We see two main steps necessary to increase the level of specificity in mitochondrial science. The first is to deliberately avoid using the terms ‘mitochondrial function’ and ‘mitochondrial dysfunction’. This admittedly uncomfortable exercise forces even the most seasoned mitochondrial investigators towards

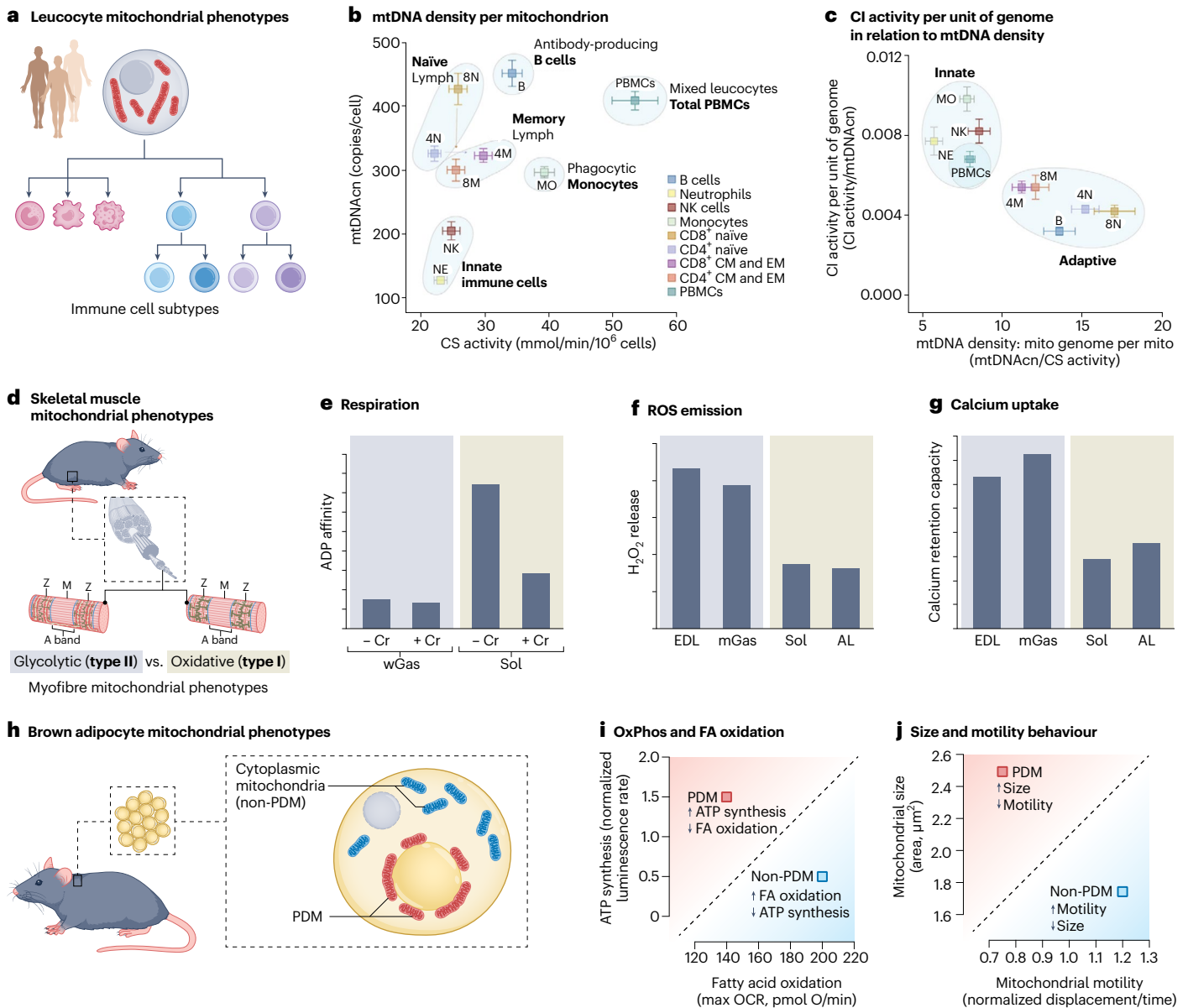


Fig. 2 | Different cell types and subcellular compartments contain functionally specialized mitochondrial phenotypes. **a**, Despite originating from the same progenitor haematopoietic stem cell, differentiated human circulating leucocyte subtypes exhibit distinct mitochondrial content and respiratory chain properties. **b, c**, Bivariate plots illustrating quantitative differences in mtDNAcn, CS activity and OxPhos complex I enzymatic activity. Data for eight different human cell subtypes, plus the heterogeneous cell mixture in peripheral blood mononuclear cells (PBMCs), were isolated using FACS. Data are from ref. 59. Note the clustering of similar mitochondrial phenotypes according to known immunological and immunometabolic differences between naïve and memory cells, or between cell subtypes belonging to the innate and adaptive arms. **d**, Functional differences among rat skeletal muscle mitochondria between glycolytic (type II, fast-twitch) and oxidative (type I, slow-twitch) muscle fibres. **e–g**, Three mitochondrial functions are shown: the sensitivity of respiration (**e**) to ADP concentration and presence of creatine (Cr), where a low Michaelis constant (K_m) means that high respiratory rates are driven by little ADP; ROS emission (**f**), H_2O_2 release per unit of mitochondrion indexed by CS; the total amount of Ca^{2+} uptake (**g**) that mitochondria can sustain before undergoing PTP opening. –Cr, no creatine; +Cr, with creatine; wGas, white gastrocnemius (type II); Sol, soleus (type I); EDL, extensor digitorum longus (type II); AL, adductor longus (type I). Data are from ref. 65. **h**, Mouse adipocytes contain at least two different types of mitochondria: PDM and non-PDM (cytoplasmic). **i, j**, Biplots representing (**i**) the maximal rate of ATP synthesis and maximal oxygen consumption rate (OCR); (**j**) or size versus motility. Data are from refs. 71,72. NK, natural killer.

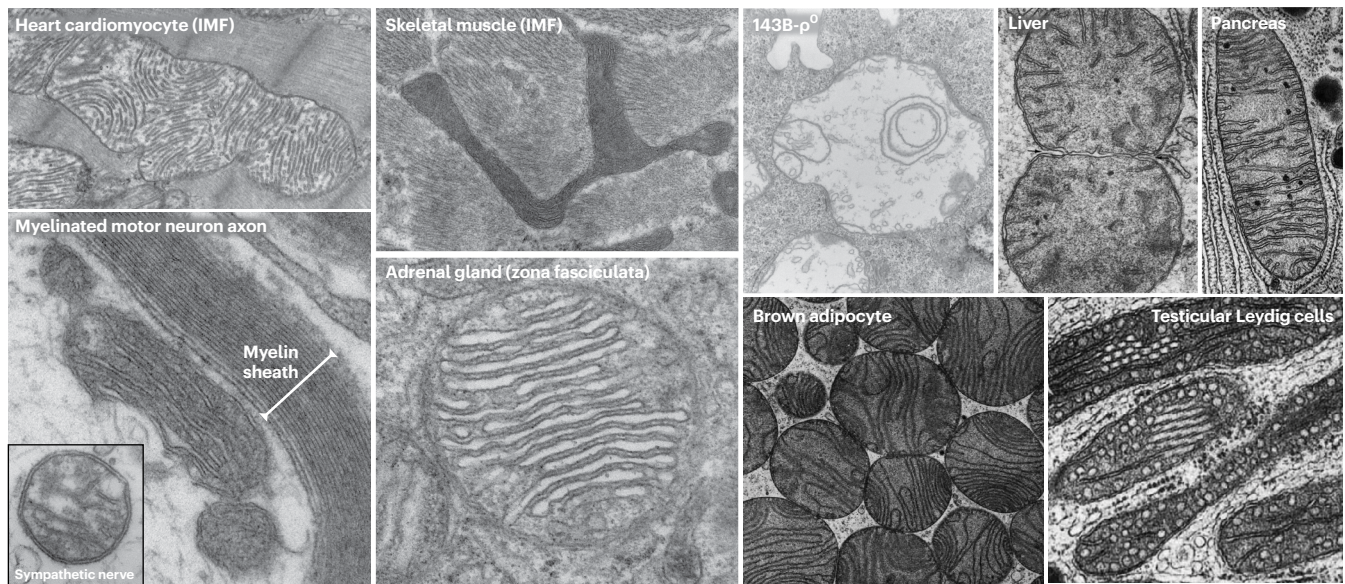
and oxidative (type I, slow-twitch) muscle fibres. **e–g**, Three mitochondrial functions are shown: the sensitivity of respiration (**e**) to ADP concentration and presence of creatine (Cr), where a low Michaelis constant (K_m) means that high respiratory rates are driven by little ADP; ROS emission (**f**), H_2O_2 release per unit of mitochondrion indexed by CS; the total amount of Ca^{2+} uptake (**g**) that mitochondria can sustain before undergoing PTP opening. –Cr, no creatine; +Cr, with creatine; wGas, white gastrocnemius (type II); Sol, soleus (type I); EDL, extensor digitorum longus (type II); AL, adductor longus (type I). Data are from ref. 65. **h**, Mouse adipocytes contain at least two different types of mitochondria: PDM and non-PDM (cytoplasmic). **i, j**, Biplots representing (**i**) the maximal rate of ATP synthesis and maximal oxygen consumption rate (OCR); (**j**) or size versus motility. Data are from refs. 71,72. NK, natural killer.

more specific terminology and a refinement of thought. It also promotes productive exchanges between experts and neophytes⁴.

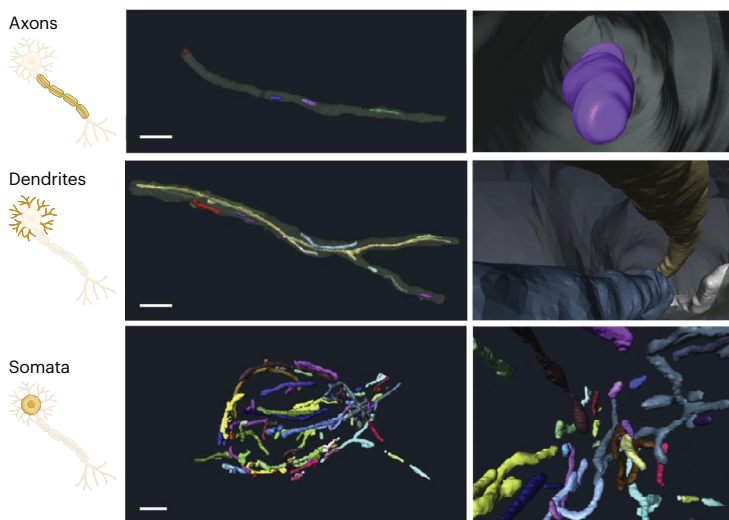
By reflexively writing ‘mitochondrial dysfunction’, do we mean a decrease in ATP synthesis rate or maximal respiratory capacity, a metabolic shift from beta-oxidation to glutamine oxidation, a decrease in OxPhos subunit abundance or supercomplex assembly, a change in ROS production, reduced mitochondrial proteostasis, a

change in membrane lipid composition, a compensatory increase in mtDNAcn, an increased cytoplasmic citrate export, or a change in motility or fusion dynamics among perinuclear mitochondria? Specificity in language begets specificity of hypotheses. In turn, clear hypotheses clarify the ideal experimental model(s) and approach(es) required to test them. Relinquishing ‘mitochondrial function’ and ‘mitochondrial dysfunction’ from our verbiage appears as a necessary,

a Morphological and ultrastructural diversity of mitochondria across mammalian tissues and cells



b Mitochondrial subpopulations in mouse brain hippocampal neurons



c Mitochondrial subpopulations in mouse and human skeletal myofibre

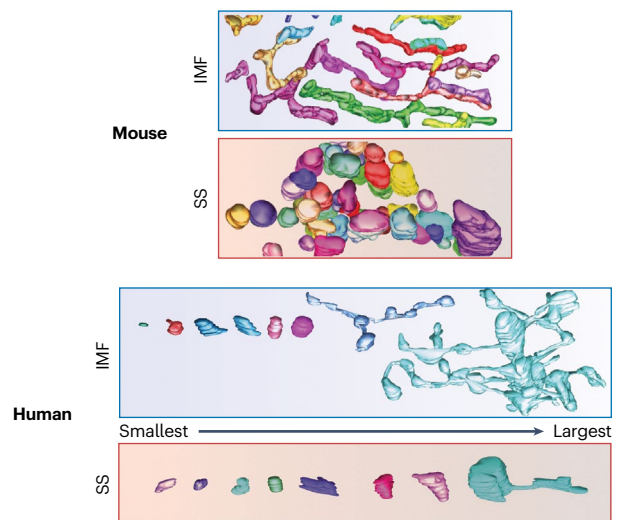


Fig. 3 | Diversity in mitochondrial morphology. **a**, TEM micrographs of mitochondria in mammalian tissues and cultured cells. The 143B- p^0 mitochondrion lacking mtDNA is from ref. 215. Adrenal mitochondrion reproduced with permission from ref. 216. Liver, pancreas, brown adipocyte and Leydig cell mitochondria reproduced with permission from ref. 73; other images are from M.P.'s laboratory). Note the natural variation in morphology (gross shape of mitochondria), in ultrastructure (positioning and organization

of internal cristae membranes) and overall electron density (reflecting density of molecular components). **b,c**, Three-dimensional reconstructions (**b**) of neural mitochondria from the subcellular compartments of large granule neurons in the mouse dentate gyrus (adapted from ref. 85), and of skeletal muscle (**c**) mitochondrial phenotypes between the SS and IMF regions of human skeletal muscle fibres (adapted from ref. 86). Note the variation in morphological complexity and volume within the mitochondrial population of the same cell.

or at least a useful, step towards a maximally productive future for the growing field.

Towards greater specificity. Without these catch-all terms, we need a systematic classification system to capture all measurable elements of mitochondrial biology. Therefore, the second step to increase specificity in mitochondrial science is to build such a system.

By analogy, medicine uses organ systems and organ-specific functions to organize medical training, diagnosis and treatment. One must naturally specify whether the electrical rhythm of the heart (electrocardiogram) or brain (electroencephalogram) is to be measured, for these provide different functional information about the organism. Similarly, cell biological processes can be examined at the levels of molecular

features that define cell types (for example, cell surface receptors and transcriptional factors), endogenous molecular activities (for example, transcription, translation and intracellular signalling), cellular functions (for example, secretion, migration and contraction) or sets of whole-cell behaviours within their environment (for example, extravasation, phagocytosis and developmentally induced programmed cell death).

Using the same hierarchical logic, we distinguish five main levels of analysis for mitochondrial biology: (1) cell-dependent mitochondrial properties, (2) static molecular features, (3) single-enzyme activities, (4) organellar functions and (5) behaviours. Figure 5 illustrates some examples of each level of analysis, which are described below starting from the smallest and most indirect, to the most complex elements of mitochondrial biology.

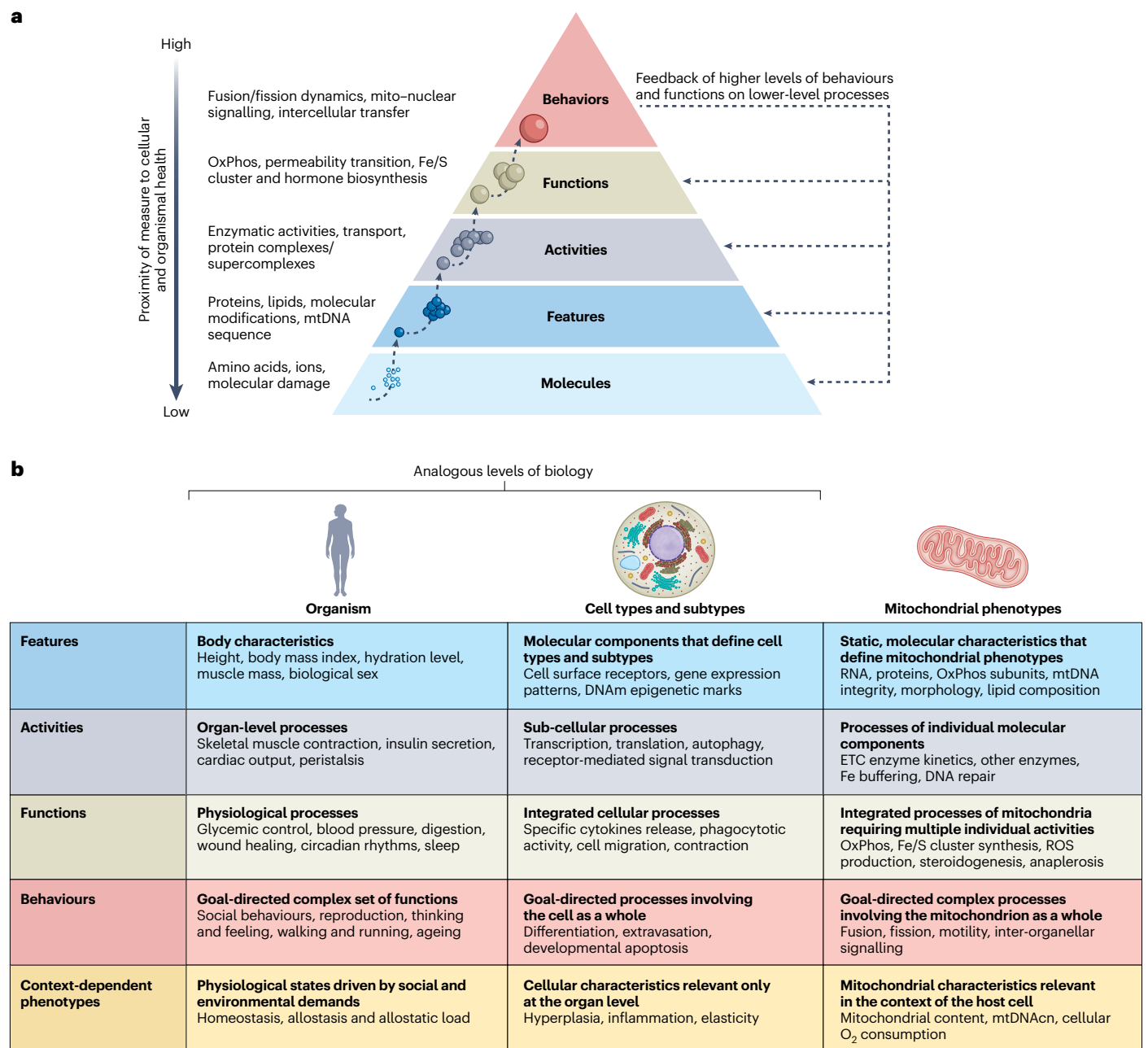


Fig. 4 | Terminology for mitochondrial science organized as a hierarchy of mitochondrial needs. **a**, Inspired by Maslow's pyramid of human needs²¹⁷, depicted is a hierarchy of biological organization from molecules to complex organellar behaviours. Lower levels combine to enable higher levels of organization. Each level can be studied using specific types of laboratory methods and analytical approaches. Thus, different approaches provide different types of information about the molecular features, activities,

functions and behaviours that define specific mitochondrial phenotypes. **b**, Operationalization and examples for different levels of organization available to examine and perturb mitochondrial biology. Biomedical terminology related to organismal characteristics (left), and terminology related to cell biology (middle) are provided as parallel illustrative examples at each level of description for mitochondrial biology.

The five main levels of analysis in mitochondrial biology

Cell-dependent mitochondrial properties. Cell-dependent mitochondrial properties are not defined based on intrinsic components of the organelle; they are meaningful only in the context of the cell. As a result, they are the most indirect as they relate to mitochondrial biology. This includes mitochondrial content or volume density, reflecting the proportion of cellular volume occupied by a given mitochondrial phenotype^{88,89}. Similarly, mtDNAcn represents the number of mtDNA copies per cell, typically expressed per copy of diploid nuclear genome. mtDNAcn varies widely between tissues, typically between ~100 to ~5,000 copies per cell^{59,90}. On its own, mtDNAcn says little to nothing

about the functional state or specialization of a mitochondrial phenotype but may be interpretable in the context of other features, activities and functions⁹¹. Other mitochondrial properties, such as the topology or network distribution of mitochondria within the cell cytoplasm and perinuclear region^{92,93}, or in specialized appendages such as presynaptic terminals⁹⁴, also bear direct functional significance, but only in the context of the cell (see Fig. 5).

Mitochondrial features. Features are the intrinsic building blocks of mitochondria. They are generally static molecular components, such as the abundance of specific proteins, membrane lipids, mtDNA

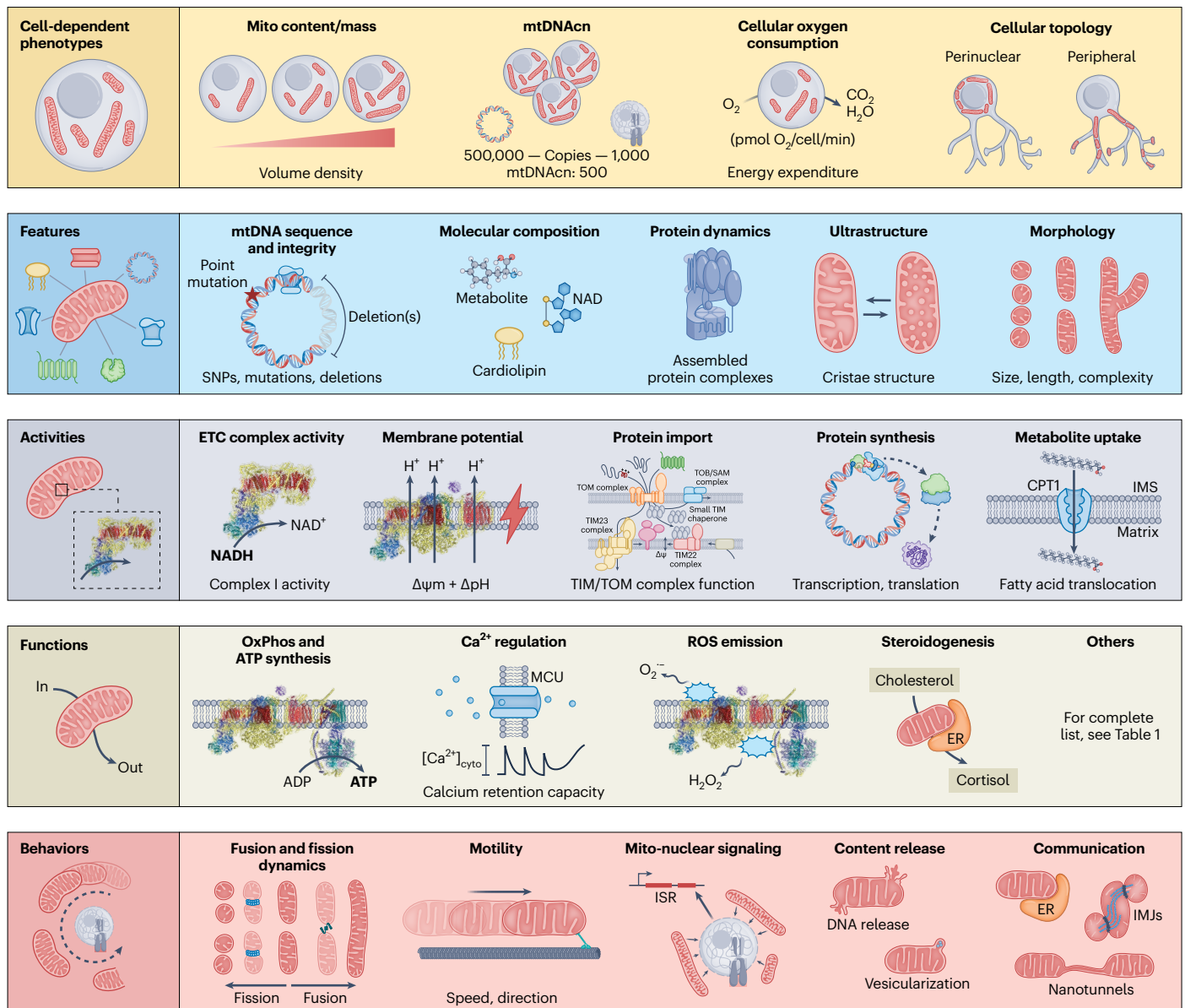


Fig. 5 | Example of measurements across domains of mitochondrial biology. Cell-dependent phenotypes: Frequently used mitochondrial measures such as mitochondrial content (also known as mitochondrial mass), mtDNAcn per cell, and OCR by cells or tissues do not reflect intrinsic mitochondrial properties. Rather, they provide information about cellular energy demand and/or cell-level regulatory processes controlling mitochondrial biology. Features: Features are molecular components that can vary in quantity or quality, generally measurable from frozen or dead cellular material. Activities: Activities emerge from the interaction of multiple features, resulting in specific enzymatic activities or intrinsic properties of mitochondria that change the effective concentration of one or more substrates. Functions: Functions emerge from the combination of several activities, resulting in the transformation of inputs into outputs at the organelle level. Example of activities include energy transformation

through the OxPhos system, Ca²⁺ regulation, macromolecule biosynthesis and the production of signals or outputs. Behaviours: Behaviours emerge from the interaction of multiple functions in collaboration with cytoplasmic and inter-organellar factors. As in cells and organisms, behaviours are best understood as goal driven, meaning that they reflect the coming together of several functions towards an end goal, such as modulating the architecture of the mitochondrial network through dynamics and motility, altering nuclear gene expression through repositioning and signalling, or optimizing cellular and organismal adaptation through inter-organellar and cell-cell communication⁷. For a list of mitochondrial functions and behaviours, see Table 1. SNP, single-nucleotide polymorphism; MCU, mitochondrial calcium uniporter; ISR, integrated stress response; IMJ intermitochondrial junctions.

integrity, the density and configuration of cristae membranes, and many other quantifiable metrics. Most omics platforms (such as proteomics, lipidomics, transcriptomics and genomics) target static features. As demonstrated in MitoCarta⁵², profiling mitochondrial features provides rich information on the molecular specialization of mitochondria (that is, the hardware). However, quantifying mitochondrial features does not reflect their functional capacity or behaviours in their cellular context. Static measures of mitochondrial morphology and

ultrastructure, which include quantitative measures of size (volume) and morphological features (length, three-dimensional morphological complexity, cristae density, and so on^{86,95}) also belong to the category of mitochondrial features.

Mitochondrial activities. Activities are single-enzyme activities that are measured as dynamic processes, such as the biochemical activity of monomeric (for example, CS) or multimeric (for example, pyruvate

Table 2 | Infusing specificity into our mitochondrial terminology can enhance how we design and communicate research

Non-specific notation	Limitation or problem	Specific notation
In a talk or conversation: A) 'We measured mitochondrial dysfunction [...].'	There are dozens of functions, and therefore dozens of ways to exhibit dysfunction.	A) General: 'We measured mitochondrial phenotypes [...].' Specific: 'We measured skeletal muscle mitochondrial OxPhos enzyme activities and citrate production [...].'
From an abstract: B) 'We assessed skeletal muscle mitochondrial function using western blot, lipidomics, biochemical assays, ATP synthesis and live cell microscopy.'	These reflect different domains of mitochondrial biology; not all are functions.	B) 'We assessed skeletal muscle mitochondrial features including protein abundance and lipid composition, OxPhos enzyme activities using biochemical assays, OxPhos function by monitoring ATP synthesis rates and mitochondrial behaviour by quantifying fusion dynamics by live cell microscopy.'
From a paper: C) 'In summary, we have studied the role of mitochondrial dysfunction in heart failure and evaluated mitochondrial boosting strategies as a potential treatment.'	Which is the actual nature of the dysfunction? What function is needed to boost?	C) 'Here, we have studied the role of endothelial cell mitochondrial functional recalibrations in heart failure and strategies to boost respiratory chain capacity as a potential treatment.'
From a grant or website: D) 'The hallmarks of ageing include telomere attrition, mitochondrial dysfunction, [...].'	In some contexts, a general qualifying term may be needed to refer to molecular, structural, functional and behavioural changes among mitochondria. This term should be plural. Not all changes reflect dysfunction but are rather adaptive recalibrations.	D) General: 'The hallmarks of ageing include telomere attrition, mitochondrial impairments and recalibrations, [...].' Specific: 'The hallmarks of ageing include telomere attrition, reduced mitochondrial OxPhos capacity, [...].'

dehydrogenase complex, PDC) enzymes. Activities are made of features but do not classify as mitochondrial functions. Mitochondrial activities include the isolated enzymatic activities of OxPhos complexes³⁶ and any other enzymatic activities, the isolated activity of individual IMM transporters like the ATP/ADP antiporter, proteases, polymerases, helicases, metabolite and ion transport across the IMM, to name a few examples.

Mitochondrial functions. Functions require at least one step to be physically localized within the mitochondrion, and generally involve multiple activities contributing to the conversion of an input into an output. ATP synthesis, Ca²⁺ homeostasis, lipid synthesis and many other processes are mitochondrial functions enabled by the interaction of two or more (often dozens) molecular features and activities, cooperating as an integrated system. For example, the conversion of electrons from reducing equivalents into an electrochemical gradient (that is, membrane potential, $\Delta\Psi_m + \Delta pH$) is considered a mitochondrial function. Similarly, protein import requires the interaction of multiple proteins and activities to transport and process proteins from the cytoplasm to the mitochondrial matrix. Some functions include complex operations that involve the collaboration of mitochondria with other organelles. A function that illustrates this

cooperativity is steroidogenesis within adrenal and gonadal mitochondria. Steroidogenesis requires the import of cholesterol from the cytoplasm to the matrix via the outer mitochondrial membrane (OMM) protein STAR, a redox-dependent side-chain cleavage reaction by the matrix P450_{ssc} enzyme, and in the case of cortisol, several steps in the endoplasmic reticulum (ER) followed by the final enzymatic step by the matrix enzyme 11-beta-hydroxylase^{34,97}. Collectively, these features and activities produce the diffusible endocrine hormone cortisol, making cortisol synthesis a proper mitochondrial function.

Fe/S cluster synthesis also involves several enzymatic and biochemical steps uniquely positioned in mitochondria. Both steroidogenesis and Fe/S cluster synthesis are mitochondrial functions essential to animal life; the former is specific to a few specialized mitochondria in the adrenal glands and gonads, while the latter is essential to the life of all eukaryotic cells. Table 1 provides a developing list of mitochondrial functions and behaviours (not including cell-dependent properties, features or activities, as they would be too numerous), which exhibit variable degrees of mitochondrial specialization across cell types and tissues.

Mitochondrial behaviours. Behaviours are best understood as a goal-driven series of activities and functions that often involve the organelle as a whole, rather than a specific set of features. Mitochondrial behaviours include motility⁶, fusion and fission dynamics⁹⁸, biogenesis⁹⁹ and mitochondrial–nuclear signalling via an array of metabolic intermediates, ions, proteins and other factors^{31,100}. For example, mitochondrial fission is a complex behaviour requiring the coordination of numerous core proteins and adaptors, in partnership with other organelles^{101,102}, whose goal is to segment individual organelles into two organelles in a non-random, functionally relevant manner¹⁰³. Like other behaviours, fission is a goal-directed set of activities that morphologically and functionally reshape the whole organelle (and in some cases, the cell itself).

The distinction between mitochondrial functions and behaviours is directly analogous to general classifications of intrinsic functions that take place within cells (gene expression, autophagy, contraction and secretion) compared to goal-directed cellular behaviours that take place within the context of other cells (infiltration, cytotoxic killing and communication). Whereas functions typically refer to processes that take place endogenously within the organelle or in a 'mitochondrial-autonomous' manner, behaviours typically involve changes or movement of the whole organelle and/or interactions with other organelle or intercellular partners.

Overcoming mitochondrial jargon

Common mitochondrial science approaches and phrases rest on the long-standing simplification that mitochondria in different cell types either 'function' or are 'dysfunctional'. The evidence reviewed above shows this to be inaccurate. Table 2 highlights some typical phrases together with their limitation, and a reframed statement offering greater specificity. The proposed framework and nomenclature system can guide the design, execution, interpretation, reporting and teaching of mitochondrial science among both experts and non-experts.

Concluding remarks

Building on previous successful efforts to create highly specific terminology and classification systems to guide scientific inquiry in medicine and in cell biology, we have outlined the beginning of such a framework for mitochondrial science. The integration of molecular, functional and morphological/ultrastructural data highlights the existence of specialized, multifaceted mitochondrial phenotypes, which exist across organs and tissues, cell types and subcellular compartments. Thus, the popular assumption that mitochondria are relatively uniform 'powerhouses' passively distributed across the body mainly to subserve energy demands is evidently limited. Rather, specialized

mitochondria coexist in a division of labour analogous to that seen among cell types and organs: different types of mitochondria share recognizable mitochondrial hallmarks, but largely subserve different, complementary roles within the organism. Building a systematic, high-resolution cartography of mitochondrial phenotypes is an open challenge for the field.

Because mitochondria are multifunctional and dynamically recalibrate their features, activities, functions and behaviours based on organismal demands and stressors, the terms ‘mitochondrial function’ and ‘mitochondrial dysfunction’ are misleading; the mitochondrial community at large would benefit from adopting more specific and informative terminology to guide teaching and thinking, our experimental designs and future theories. The terminology system outlined in Figs. 4 and 5 is pragmatic, hierarchical and measurement oriented. In the same way that cell biology has benefited from fine-grained classification systems among its subspecialties, the proposed semantic and scientific specificity should deepen our mechanistic models of the role that mitochondria play in health and disease states. Importantly, as mitochondrial science is increasingly poised to contribute meaningful knowledge to other areas of biology and medicine, the proposed classification system also is likely to maximize the interdisciplinary synergy between mitochondrial science and other disciplines.

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M.P. drafted the figures. A.S.M., J.A.E. and M.P. contributed to the literature review and revised the final version of the figures and manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to Martin Picard.

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