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The multifaceted contributions of mitochondria to cellular metabolism

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Although classically appreciated for their role as the powerhouse of the cell, the metabolic functions of mitochondria reach far beyond bioenergetics. In this Review, we discuss how mitochondria catabolize nutrients for energy, generate biosynthetic precursors for macromolecules, compartmentalize metabolites for the maintenance of redox homeostasis and function as hubs for metabolic waste management. We address the importance of these roles in both normal physiology and in disease.

The transition to a highly oxidizing atmosphere in early Earth development created selective pressure that favoured organisms with respiratory capacity[1,2], including heterotrophic anaerobes, which consumed aerobic prokaryotic microorganisms (protomitochondrion)[3]. Following endosymbiosis, mitochondrial signals have been synchronized with the eukaryotic cell[4]. This integral relationship is demonstrated by the compartmentalized nature of cellular metabolism, in which mitochondrial reactions are required components of metabolic pathways.

Mitochondria coordinate cellular adaptation to stressors such as nutrient deprivation, oxidative stress, DNA damage and endoplasmic reticulum (ER) stress[5]. Although long known to be critical for bioenergetics, emerging research shows that mitochondrial metabolism is multifaceted, mirroring their diverse functions. In addition to ATP, mitochondria produce metabolic precursors for macromolecules such as lipids, proteins, DNA and RNA. Mitochondria also generate metabolic by-products, such as reactive oxygen species (ROS) and ammonia, and possess mechanisms to clear or utilize waste products.

In this Review, we discuss the metabolic functions of mitochondria as bioenergetic powerhouses, biosynthetic centres, balancers of reducing equivalents and waste management hubs. Metabolic compartmentalization is instrumental for mitochondria to perform these functions. We highlight how mitochondrial metabolism supports their diverse functions in cell biology and how metabolism is compartmentalized in normal physiology and disease. A deeper understanding of mitochondrial contributions to metabolism will further elucidate their roles in disease and may reveal co-dependent pathways to target in therapies.

Mitochondria are the powerhouses of the cell

Cells consume fuels such as sugars, amino acids and fatty acids to generate energy in the form of ATP and GTP (ref. [6]). Nutrients are metabolized and shuttled into the tricarboxylic acid (TCA) cycle, and through iterative oxidations, electrons are stored in the reducing equivalents NADH and $FADH_2$ (ref. [6]). These carriers deposit electrons into the electron transport chain (ETC) in the inner mitochondrial membrane (IMM), and use electron flow to pump protons into the intermembrane space[7]. Protons flow down their electrochemical gradient through F_1F_0–ATP synthase to generate ATP (ref. [8]). Although oxidative phosphorylation is the largest source of cellular ATP, the potential energy generated by the ETC is also harnessed for biosynthetic purposes. Many diseases arise when the ETC is perturbed[9,10]. Here, we discuss how mitochondria integrate fuel metabolism to generate energy for the cell, encompassing both classical and unconventional fuel sources (Fig. 1).

Pyruvate. Pyruvate is generated by a number of sources, depending on nutrient availability and tissue, including glucose catabolism (thought to be a major source) and lactate[11–13]. Pyruvate utilization in the cytosol versus mitochondria is one of the clearest examples of how compartmentalization is a major determinant of cellular bioenergetics. In healthy tissue, the fate of pyruvate is dependent on oxygen availability and mitochondrial respiratory capacity[14]. In normoxia, pyruvate is generated through glycolysis and transported across the IMM through the mitochondrial pyruvate carrier (MPC)[15,16]. Pyruvate is further catabolized inside mitochondria through the TCA cycle. During hypoxia, mitochondrial respiration is repressed, causing cells to adaptively sink electrons onto pyruvate through lactate dehydrogenase (LDH), generating lactate in the cytosol[17]. This pathway is engaged in muscle during exercise, the intestines and the renal medulla of the kidneys[18–20]. Otto Warburg observed that cancer cells rewire glucose metabolism for lactate synthesis even in normoxia, known as the Warburg effect[14,21]. Additional studies must be performed to determine the net catalytic activity of LDH in tumours, given that metabolic tracing studies in lung cancer patients have demonstrated that lactate is a major source of TCA cycle intermediates[13]. The extent of LDH-mediated pyruvate production may depend on in vitro versus in vivo models of tumour metabolism, emphasizing the need to test metabolic flux in vivo.

The critical role of pyruvate compartmentalization in bioenergetics and metabolism is highlighted by recent elegant studies of the MPC (refs [15,16]). Pharmacological inhibition of MPC represses mitochondrial pyruvate uptake, shifting reliance to glycolysis for ATP production. This shift is evident in cancer cells, which repress MPC1 to promote the Warburg effect, and in myocytes of diabetic mice, which elevate glucose consumption in response to MPC inhibition[22,23]. Suppression of MPC accelerates proliferation in intestinal stem cells[24], suggesting that the role of MPC is context-dependent and sensitive to mitochondrial respiratory capacity and/or nutrient availability.

Within mitochondria, pyruvate may enter the TCA cycle through the activity of two distinct enzymes: pyruvate dehydrogenase complex (PDC), which generates acetyl CoA, and pyruvate carboxylase (PC), which generates oxaloacetate[25]. Although PDC and PC both catalyse the flux of pyruvate into the TCA cycle, their enzymatic activities can be distinguished by stable isotope tracing[26,27], and their metabolic roles do not appear to be interchangeable. PDC deficiency is sufficient to rewire energy metabolism towards aerobic glycolysis despite the potential adaptive node for TCA cycle anaplerosis (a process to replenish TCA cycle intermediates), mediated by PC (ref. [28]). Many cancers favour PC-mediated anaplerosis, although

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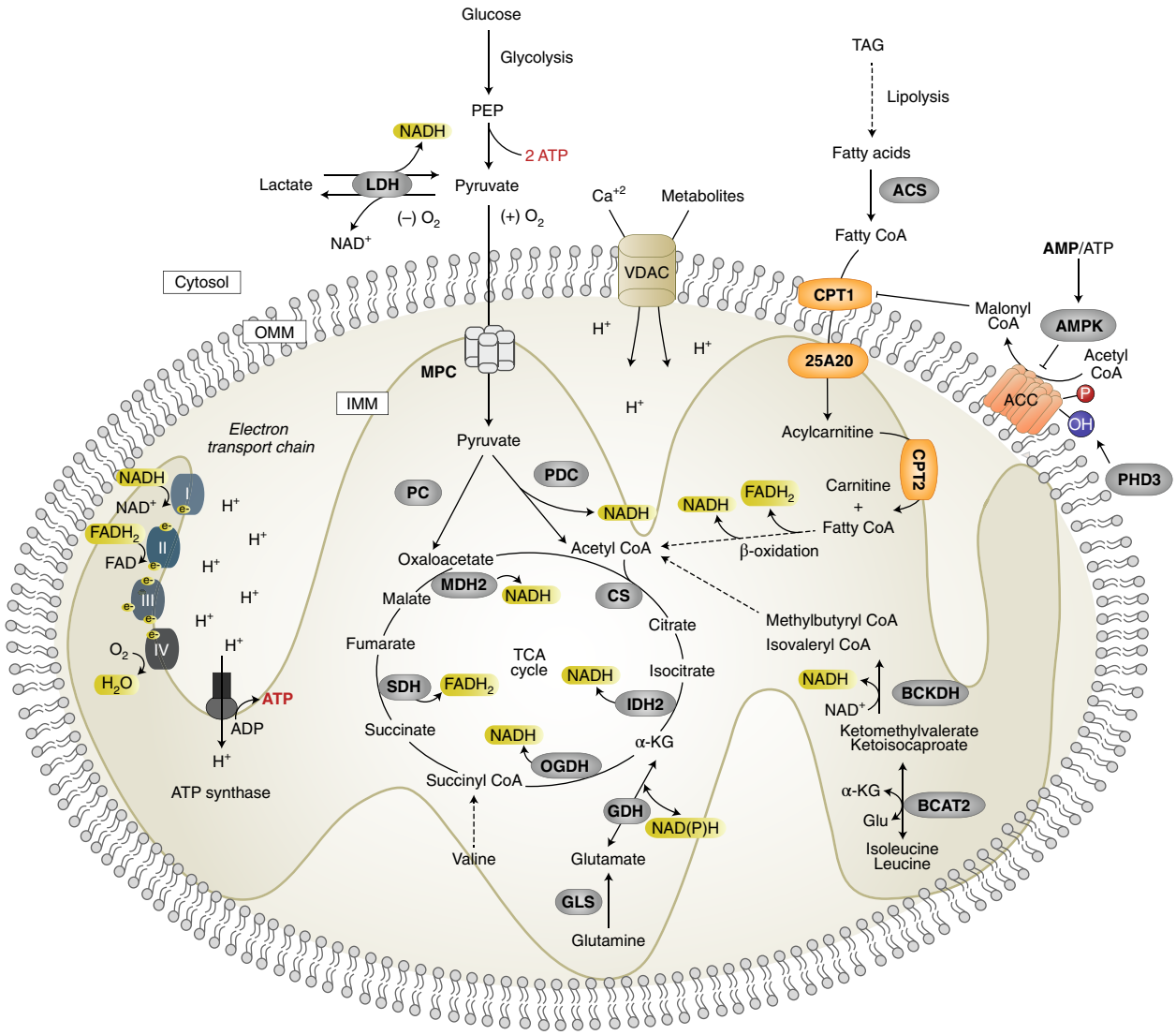

Fig. 1 | Mitochondria are the powerhouse of the cell. Mitochondria integrate fuel metabolism to generate energy in the form of ATP. Mitochondria oxidize pyruvate (derived from glucose or lactate), fatty acids and amino acids to harness electrons onto the carriers NADH and $FADH_2$. NADH and $FADH_2$ transport these electrons to the electron transport chain, in which an electrochemical gradient is formed to facilitate ATP production through oxidative phosphorylation. VDAC, voltage-dependent anion channel; IDH2, isocitrate dehydrogenase 2; OGDH, α-ketoglutarate dehydrogenase; SDH, succinate dehydrogenase; BCAT2, branched-chain amino transferase 2; ACS, acyl CoA synthetase. Electrons and reducing equivalents are shown in yellow.

the factors that dictate the choice for pyruvate-flux between PC and PDC are little studied[27,29,30]. Therefore, these enzymes may have important functions beyond TCA-cycle-flux for bioenergetics.

Glutamine and branched-chain amino acids. Catabolism of glutamine, the most abundant amino acid in plasma, often starts in the mitochondria, and its carbon and nitrogen atoms are distributed into macromolecules (DNA, RNA, protein and lipids) and other metabolites, such as TCA cycle intermediates (important in bioenergetics), amino acids, nucleotides and glutathione[31].

In mitochondria, glutaminase (GLS) converts glutamine into glutamate and ammonia. Either transaminase or glutamate dehydrogenase (GDH) converts glutamate into α-ketoglutarate (α-KG)[32,33]. Glutamine anaplerosis sustains TCA cycle intermediates in conditions of limited glucose and MPC inhibition, demonstrating the potential flexibility of these metabolic nodes[34,35]. Glutamine anaplerosis is critical for meeting the energetic requirements of proliferative cells, such as T cells during the transition from quiescent naïve T cells to effector cells, and in cancers, particularly those with MYC elevation[32,36,37]. GLS inhibition suppresses proliferation, and GLS inhibitors are being evaluated in clinical studies for a number of cancers[31,38,39]. However, sensitivity to GLS inhibition in vitro is not always consistent in vivo, and is dependent on extracellular cystine levels[40]. This emphasizes the need for investigators to study the effect of the microenvironment on metabolic dependencies and to validate experiments in vivo.

Although glutamine transporters at the plasma membrane have been identified[41], the mitochondrial glutamine transporter has not been fully characterized[42,43]. This critical area of research is challenging to address because there are likely multiple mechanisms for glutamine import.

The branched-chain amino acids (BCAAs) leucine, isoleucine and valine are major sources of cellular energy through generation of acetyl CoA and succinyl CoA (ref. [44]). The tissue of origin dictates dependency on BCAA catabolism in normal physiology and in cancer[45]. In normal physiology, myocytes and adipocytes activate mitochondrial BCAA catabolic enzymes to support ATP production during exercise or fasting, and during differentiation, respectively[46,47].

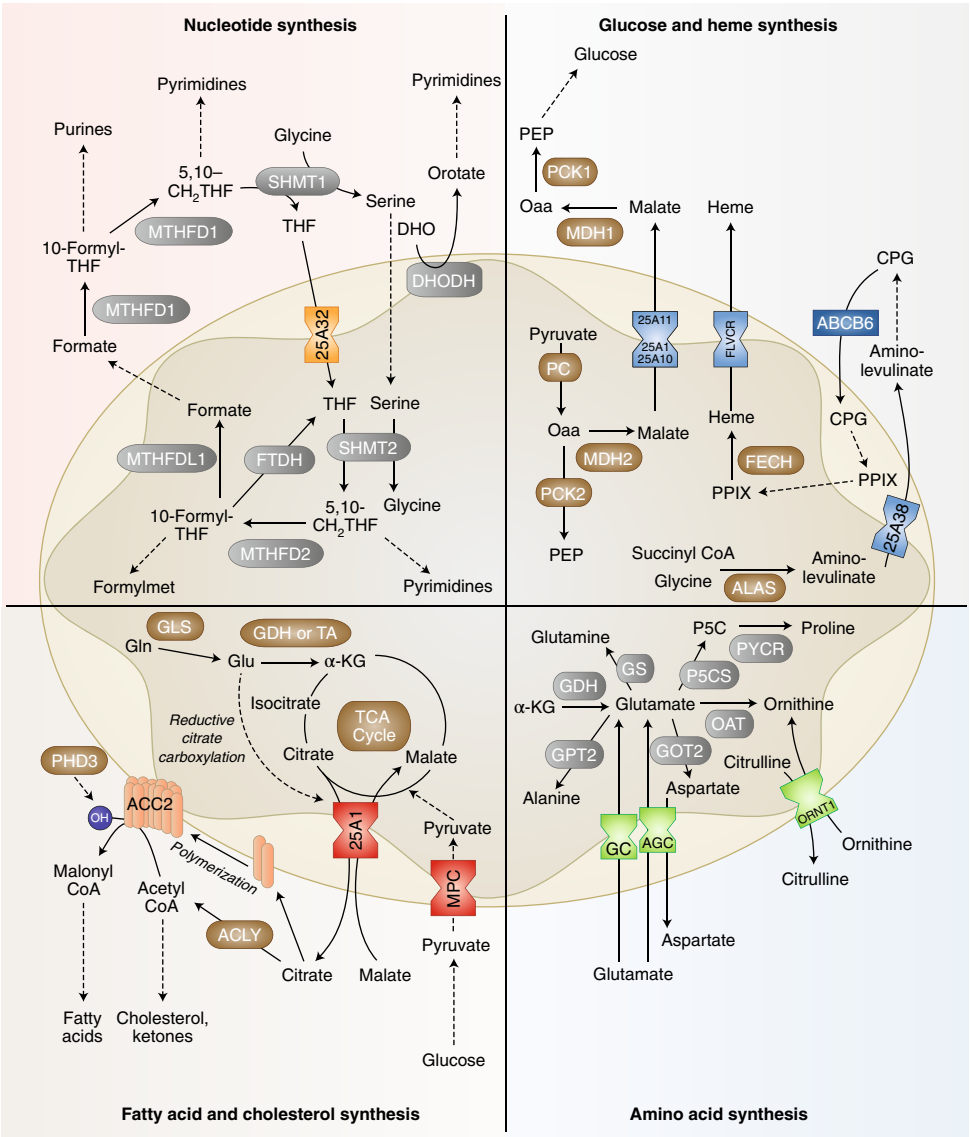

Fig. 2 | Mitochondria are biosynthetic hubs. The mitochondria are a critical source of building blocks for biosynthetic pathways, including nucleotide synthesis, fatty acid and cholesterol synthesis, amino acid synthesis, and glucose and heme synthesis. Compartmentalization is a key feature of biosynthetic pathways. While many of the enzymes listed are bi-directional, arrows highlight the biosynthetic functions. Enzymes are circled in grey and brown. FTDH, formate dehydrogenase; TA, transaminase; GC, glutamate carrier; FLVCR, feline leukaemia virus subgroup C receptor 1.

BCAA catabolism is repressed in maple syrup urine disease, which is caused by mutations to branched-chain keto acid dehydrogenase (BCKDH) and causes dysfunction of immune cells, skeletal muscle and the central nervous system[48]. Although mitochondrial BCAA catabolism is critical in these pathologies, it is unknown how BCAAs are imported into the mitochondria. Identifying their transport mechanisms will be critical to our understanding of mitochondrial BCAA catabolism in cellular homeostasis.

Fatty acid oxidation. Palmitate, a 16-carbon fatty acid (FA), stores 39 KJ g^{-1} of energy, compared to 16 KJ g^{-1} stored in glucose[49]. Therefore, FAs are a major source of cellular energy, particularly under conditions of nutrient stress. Mitochondrial FA import is a rate-determining step for fatty acid oxidation (FAO) and demonstrates how metabolic compartmentalization adapts to cellular state. As long-chain FAs are unable to cross mitochondrial membranes, mitochondria have evolved an intricate set of reactions and transporter activities to allow fat to access mitochondrial β-oxidation machinery. The outer mitochondrial membrane (OMM) enzyme carnitine palmitoyl transferase 1 (CPT1) forms acylcarnitines from fatty acyl CoAs[50]. Acylcarnitines are shuttled into mitochondria through the carnitine–acylcarnitine translocase (SLC25A20) in the IMM. CPT2 liberates FA from carnitine, initiating FAO[51]. Acetyl CoA from FAO is used for the TCA cycle as well as for aspartate and nucleotide synthesis[52].

CPT1 activity is tightly controlled by a network of metabolites, linking it to cellular nutrient status. Malonyl CoA, generated by the enzyme acetyl CoA carboxylase (ACC), represses CPT1 to inhibit acylcarnitine import[53]. Malonyl CoA is the initiating metabolite for FA synthesis, and its levels dictate the balance of fat synthesis or oxidation within a cell. In low-energy conditions, AMP-activated protein kinase (AMPK) phosphorylates and inhibits ACC, decreasing malonyl CoA and increasing CPT1 activity[54]. ACC2 is also hydroxylated by the dioxygenase prolyl hydroxylase 3 (PHD3)[55]. Hydroxylation promotes ACC2 activity in nutrient abundance. These enzymes are altered in some cancers and human diseases as

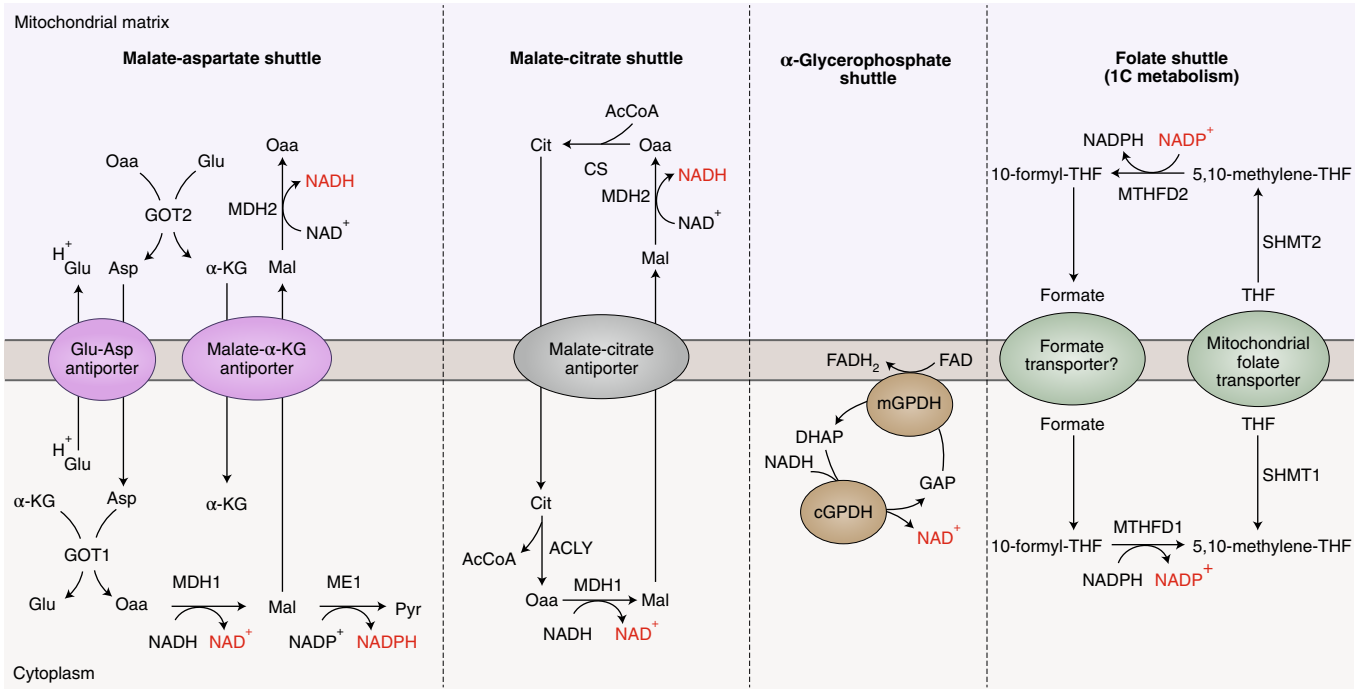

Fig. 3 | Mitochondria balance redox equivalents. In the absence of a direct mode for NAD transport, cells rely on compartmentalized flux of metabolites to support balance of reducing equivalents NAD+/NADH and NADP/NADPH. Generally, redox shuttles favour cytosolic NAD+ synthesis and mitochondrial NADH synthesis. ME1, malic enzyme 1; mGPDH/cGPDH, mitochondrial/cytosolic glycerol-3-phosphate dehydrogenase, respectively.

the mechanism that dictates fat utilization. PHD3 is suppressed in cancers that rely on FAO, such as acute myeloid leukaemia (AML) and prostate cancer, and elevated in cancers that rely on FAS such as breast and non-small-lung-cell cancer[55–57]. Reciprocally, AMPK is linked to fat utilization in metabolic diseases and cancers[58,59].

The dynamic regulation of FAO is key to cellular physiology. FAO is fundamental for the survival and function of memory CD8+ T cells, unlike effector cells that rely on glycolysis and glutaminolysis for energy[60,61]. Likewise, FAO is activated in insulin resistance, in which free fatty acids provide a compensatory fuel source when glucose uptake is repressed[62,63].

Mitochondria are biosynthetic hubs

Mitochondria participate in the biosynthesis of nucleotides, FAs, cholesterol, amino acids, glucose and heme (Fig. 2)[64]. These biosynthetic pathways are engaged in stress responses, and are often mis-regulated in disease[5]. Rather than being dysfunctional, highly proliferative cells, such as cancer cells and activated T cells, rely on mitochondrial metabolites to form biomass[5,65]. In the following sections, we review the mitochondrial compartmentalization of anabolic pathways and its role in cell stress responses and disease.

Nucleotides. The one carbon (1 C) metabolic pathway involves a set of reactions that generate and transfer activated 1 C units for de novo nucleotide synthesis, compartmentalize amino acids, and contribute to redox homeostasis. The co-factor tetrahydrofolate (THF) is the carrier that mediates 1 C transfer reactions for de novo nucleotide synthesis[66,67]. In this pathway, activated THF molecules are generated through an oxidative/reductive cycle that catabolizes serine (to generate glycine) in the mitochondria and synthesizes serine in the cytosol.

The carrier SLC25A32 imports THF into the mitochondria, where it is converted by serine hydroxymethyltransferase (SHMT2) into 5,10 methylene-THF and glycine. Like many enzymes in 1 C metabolism, SHMT2 is bi-directional. SHMT2 favours production of glycine and 5,10 methylene-THF, and cells deficient in mitochondrial 1 C metabolism are glycine auxotrophs[68]. In the absence of SHMT2, cytosolic SHMT1 reverses flux to compensate[69], demonstrating how metabolic flexibility among subcellular compartments is critical to stress adaptation.

Mitochondrial methylenetetrahydrofolate dehydrogenase (MTHFD2) converts 5,10 methylene-THF to 10-formyl-THF. MTHFD2 expression is regulated by mTORC1, and is critical for growth and proliferation[70]. MTHFD2 is overexpressed in many human cancers[71], and mitochondrial biogenesis and SHMT2/MTHFD2 expression are promoted during T-cell activation to support proliferation[72]. 10-formyl-THF has multiple fates: conversion into THF by 10-formyl-THF dehydrogenase; production of formylmethionine for mitochondrial translation; or hydrolysation to formate by MTHFD1L. Mitochondrial contributions to this pathway are critical, as mitochondrial formate is the main carbon source for cytosolic 1 C metabolism[66].

The IMM enzyme dihydroorotate dehydrogenase (DHODH), which oxidizes dihydroorotate to orotate, is required for de novo pyrimidine synthesis[73]. Consistent with their reliance on 1C metabolism, T cells require DHODH for clonal expansion and differentiation into effector cells[74]. DHODH is targeted in autoimmune disorders, and its inhibition suppresses myeloid differentiation of AML cells[75]. DHODH activity is also elevated in response to DNA damage, and on genotoxic chemotherapy treatment to increase nucleotide synthesis for DNA repair[76,77].

Citrate. In addition to generating electron carriers for the ETC, the TCA cycle intermediates, such as citrate, regulate anabolic reactions. Mitochondrial citrate controls anabolic reactions by directly acting as the carbon source for FAs, cholesterol and ketone bodies through ATP citrate lyase (ACLY)[78] and by allosteric modulation. Citrate is generated by citrate synthase (CS) or through the reduction of α-KG by isocitrate dehydrogenase (IDH)[79–81]. Mitochondrial citrate is exported by the malate-citrate antiporter, SLC25A1 (ref. [82]).

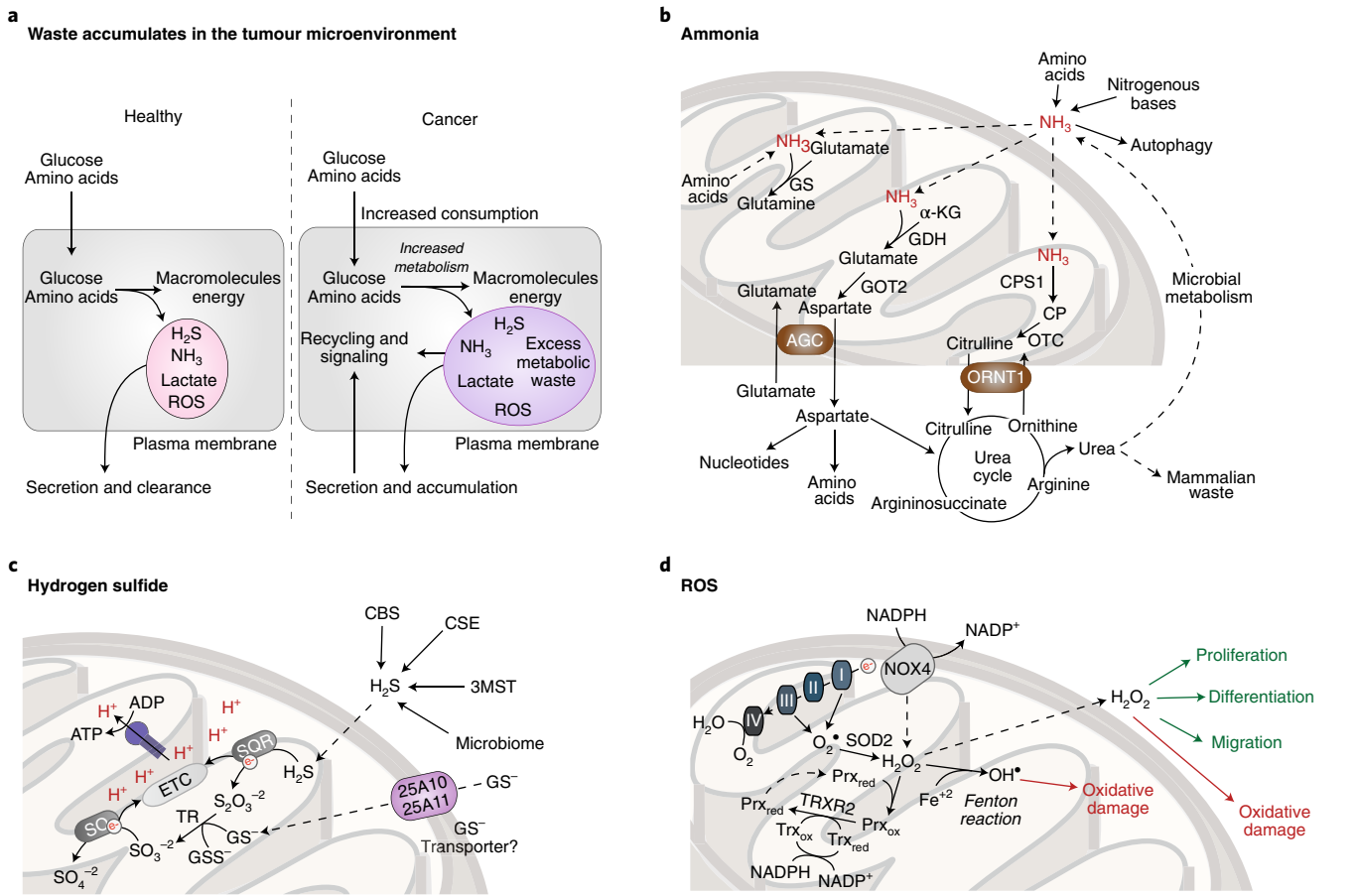

Fig. 4 | Mitochondria orchestrate waste management. a, Tumour cells increase nutrient consumption and metabolic fitness relative to healthy tissue, leading to accumulation of waste products in the tumour microenvironment. To manage metabolic waste, cancer cells engage recycling pathways for these metabolic by-products. **b**, Production and metabolic clearance of ammonia (NH_3) in cell metabolism. NH_3 is generated by amino acid and nucleotide catabolism. NH_3 is assimilated in the mitochondria through GS, GDH, and CPS1. CPS1 initiates the urea cycle for production of the metabolic waste product urea. Urea can be re-catabolized by urease positive bacteria in the microbiome to regenerate NH_3. **c**, Production and metabolic clearance of hydrogen sulfide (H_2S) in cell metabolism. H_2S is generated by the mammalian enzymes CBS, CSE, 3MST and from the metabolic reactions in the microbiome. H_2S is cleared by iterative oxidation catalysed by SQR, TR, and SO. TR utilizes oxidized glutathione (GS^-) as a sink for electrons. Oxidations catalysed by SQR and SO are linked to mitochondrial ETC and oxidative phosphorylation. **d**, Reactions that generate and sequester ROS. ROS are generated in the mitochondria through the ETC and NOX4. SOD2 converts superoxide into the less reactive molecule hydrogen peroxide (H_2O_2). In the mitochondria, H_2O_2 is turned over by combined functions of peridoxins (Prx) and thioredoxins (Trx). H_2O_2 also reacts with Fe^{+2} (the Fenton reaction) to generated $OH^•$ in the mitochondria. ROS inflict oxidative damage to proteins in the mitochondria and cytosol, and also function as potent mitogen signalling agents.

In the cytosol, citrate is converted to acetyl CoA by ACLY, which can access several pathways, including conversion to malonyl CoA by the activity of ACC (as described above in 'Fatty acid oxidation'). Cytosolic citrate is a potent allosteric regulator of ACC, increasing its polymerization and activity[83].

Regulation of citrate export may provide a physiological node for the cell to communicate lipid homeostasis to the mitochondria. SLC25A1 is sensitive to membrane rigidity, and high levels of cholesterol or acidic phospholipids in the IMM repress mitochondrial citrate export[84]. Moreover, fasting causes a 40% reduction in mitochondrial citrate export[85]. Although these studies indicate that citrate export is affected by lipid abundance, it is unknown if repression of SLC25A1-mediated citrate export affects ACC2 polymerization and FAS initiation.

Acetyl CoA is required for epigenetic modifications, such as histone acetylation[86–88]. Thus, fat metabolism may be intimately linked with the epigenetic state, although it is unknown whether the connection is direct. The emerging role of mitochondrial metabolism in epigenetic reprogramming may extend beyond acetyl CoA to include other mitochondrial metabolites such as succinate, fumarate and ROS, which directly affect the activity of Fe (II)/α-KG-dependent dioxygenases, including hydroxylases, DNA demethylases and histone demethylases[89].

Amino acids. The mitochondria are a hub for amino acid synthesis, including glutamine, glutamate, alanine, proline and aspartate. Glutamine synthetase (GS) condenses glutamate and ammonia to make glutamine[90]. GS has been reported to have activity in the cytosol and mitochondria, and its biological role may differ depending on its subcellular localization. GS has a 'weak' mitochondrial localization sequence and is imported into the mitochondria in the liver, whereas GS is cytoplasmic in astrocytes[91]. In glioblastoma, GS generates a source of glutamine for de novo purine synthesis[92]. However, in breast cancer cells, GS-derived glutamine is not used for de novo nucleotide synthesis[93]. One possible explanation for this difference is the subcellular localization of GS in these systems.

Glutamate is generated by and utilized as a nitrogen source for numerous reactions[94]. Glutamate metabolism stratifies in proliferating and quiescent cells; proliferating cells elevate the expression of glutamate-dependent transaminases, whereas quiescent cells

suppress them[95]. Many of the glutamate-dependent transaminases, such as glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), have two (cytosolic and mitochondrial) isoforms[95]. It will be key for future studies to elucidate the role of subcellular compartmentalization of glutamate metabolism in proliferation.

Proline and ornithine metabolism are centrally mitochondrial. The mitochondrial enzyme pyrroline-5-carboxylate synthase (P5CS) generates pyrroline-5-carboxylate (P5C), which can be used for proline and ornithine production[96]. Ornithine is made by ornithine amino transferase (OAT) and proline is produced through reduction of P5C by pyrroline-5-carboxylate reductase (PYCR). The mechanisms underlying compartmentalization of proteinogenic amino acids, such as proline and glutamate, are little studied[97].

Gluconeogenesis. Gluconeogenesis is predominantly a cytosolic process, although the initiating step by PC occurs inside the mitochondria[98]. PC-derived oxaloacetate is converted to malate and exported from the mitochondria for the remaining steps of gluconeogenesis[99]. This export can occur through SLC25A1 (a citrate-malate antiporter), SLC25A11 (an α-KG–malate antiporter) or SLC25A10 (a dicarboxylate-phosphate antiporter)[100]. The dominant mechanism for malate export in gluconeogenesis is unknown. Furthermore, it is unclear if metabolic stressors such as nutrient deprivation or hypoxia dictate this mechanism. In the cytosol, phosphoenolpyruvate carboxykinase (PCK) converts oxaloacetate into phosphoenol pyruvate (PEP) for gluconeogenesis[101]. The mitochondrial isoform of this enzyme, PCK2, has no known connections to gluconeogenesis[101].

Heme. Heme metabolism illustrates an extraordinary example of metabolic compartmentalization. The committed step of the pathway is catalysed by mitochondrial aminolevulinate synthase (ALAS), which generates ALA from glycine and succinyl CoA (ref. [102]). ALA is exported by SLC25A38 and, through four cytosolic reactions, is converted into coproporphyrinogen III (CPGIII). Next, CPGIII enters the intermembrane mitochondrial space through the ATP-dependent transporter ABCB6 for further catalysis by coproporphyrinogen oxidase (CPOX)[103]. The intermembrane space is a region in which few metabolic reactions occur. The terminal step of heme synthesis is in the mitochondrial matrix, in which ferrochelatase (FECH) catalyses the insertion of ferrous iron into the macrocycle[104]. As heme biosynthesis generates H_2O_2 in the intermembrane region, we speculate that there may be direct links between heme metabolism and ROS-sensitive signalling pathways.

Mitochondria balance redox equivalents

The mitochondria and cytosol have distinct requirements for NAD^+, and proper compartmentalization of redox equivalents is crucial for maintenance of cellular homeostasis and survival in response to environmental stressors[105–107]. The cytosol is a more oxidizing environment in which the NAD^+/NADH ratio ranges between 60 and 700 (ref. [108]). Conversely, mitochondria employ more reductive metabolic reactions, and the NAD^+/NADH ratio is approximately 7–8 (ref. [108]). To sustain the imbalanced distribution of NAD, mammalian cells engage indirect pathways (Fig. 3), as there is no known mammalian transporter for NAD^+ or NADH; contrary to yeast, which facilitate NAD transport through NDT1 (ref. [109]).

Malate–aspartate shuttle. The malate–aspartate shuttle is ubiquitously engaged to generate cytosolic NAD^+ and mitochondrial NADH[110]. This cycle involves an oxidation or reduction catalysed by malate dehydrogenase (MDH1, cytosolic; MDH2, mitochondrial), a transamination catalysed by glutamate–oxaloacetate transaminase (GOT1, cytosolic; GOT2, mitochondrial) and two antiporters localized to the IMM (aspartate–glutamate antiporter, AGC; malate–α-KG antiporter, MαA)[111]. Compartmentalization of reducing equivalents through the malate–aspartate shuttle is key for survival in stress conditions such as exercise, in which cytosolic NAD^+ is required to promote glucose catabolism and mitochondrial NADH for ATP production[112]. Moreover, in PDAC cancers with oncogenic KRAS, glutamine is fluxed through the malate-aspartate shuttle to raise the NADPH/$NADP^+$ ratio for glutathione synthesis[113]. In addition to its regulation of redox balance, the malate–aspartate shuttle may also contribute to cellular amino acid compartmentalization. When oxidative phosphorylation is repressed, cells utilize the reverse flux of GOT1 to generate cytosolic aspartate[114,115].

Citrate–malate shuttle. In contrast to the malate–aspartate shuttle, the citrate–malate shuttle functions equally (with respect to reducing equivalents), but is less studied in the context of disease. Similar to the malate–aspartate shuttle, the citrate–malate shuttle utilizes both isoforms of MDH. However, MDH activity is paired with CS, ACLY and the malate–citrate antiporter (CIC)[116]. Rather than elevating cytosolic aspartate, the citrate–malate shuttle increases cytosolic citrate levels. Therefore, flux through the citrate–malate shuttle promotes FAS through citrate compartmentalization[117]. Thus, although both the malate–aspartate and citrate–malate shuttles balance reducing equivalents through MDH activity, these shuttles are not interchangeable. The implications of cytosolic citrate accumulation in the malate–citrate shuttle are yet to be defined beyond FAS. For example, flux through the citrate–malate shuttle may also affect epigenetics through ACLY activity and acetyl CoA production[88].

α-Glycerophosphate shuttle. The α-glycerophosphate shuttle is a unique redox-balancing pathway, which intersects the mitochondria but does not directly affect mitochondrial NAD^+/NADH[118]. The α-glycerophosphate shuttle is composed of cytosolic and mitochondrial α-glycerophosphate dehydrogenase (cGPDH and mGPDH, respectively). In this cycle, cGPDH utilizes NADH to reduce dihydroxyacetone phosphate (DHAP) to glycerophosphate (GAP) and generate cytosolic NAD^+. GAP is subsequently oxidized to DHAP by the flavin-dependent mGPDH, which directly deposits electrons into the ETC. The α-glycerophosphate shuttle is tightly linked to glycolysis and is highly active in brown adipose tissue (BAT) to regenerate cytosolic NAD^+, while simultaneously sinking electrons into the ETC for thermogenesis[118]. As this pathway is engaged in highly glycolytic cells, it would be interesting for future studies to investigate the potential role of this redox shuttle in cancer.

One carbon metabolism. MTHFD is among the largest contributors to cellular NADPH, in addition to the pentose phosphate pathway and malic enzyme (ME)[119]. MTHFD isozymes are bi-directional, however, stable isotope tracing of NADPH revealed that the mitochondrial MTHFD favours NADPH production, and the cytosolic isoform favours $NADP^+$ production[120]. The one carbon (1 C) metabolic pathway is an adaptive mechanism to survive oxidative stress. Following ETC inhibition, flux through the mitochondrial arm of 1 C metabolism is activated for NADPH/$NADP^+$ balance[121]. NADPH is required for reduction of glutathione and clearance of ROS. In cancer cells, flux through the mitochondrial 1 C pathway generates cytosolic NADPH for FAS (ref. [122]).

Mitochondria orchestrate waste management

The by-products of metabolic reactions are often depicted as waste. However, emerging studies have revealed a functional role for metabolic by-products such as lactate, ammonia, ROS and hydrogen sulfide (H_2S)[12,13,93,123,124]. The study of metabolic by-products is a growing area of research — especially in cancer, in which metabolic by-products accumulate in the tumour microenvironment (TME)[125] (Fig. 4a). Mitochondria are indispensable in cellular waste

management (Fig. 4b–d); in this section we review the pathways that mitochondria utilize to re-purpose cellular waste.

Ammonia. Ammonia is generated in mammalian cells by amino acid lyases and nucleotide deaminases, however, the largest contributor to ammonia in mammals is the microbiome[126]. Ammonia is a neurotoxin that is sustained below 50 μM in the plasma of healthy adults, and can induce seizures when plasma levels become elevated[122]. Moreover, high ammonia may induce autophagy in some cultured cells[127,128]. To evade toxicity, mammalian cells possess three ammonia-assimilating enzymes: carbamoyl phosphate synthetase 1 (CPS1), GS and GDH.

The urea cycle is a sink for ammonia, ultimately generating urea, which cannot be metabolized by mammalian enzymes. CPS1 is the rate-limiting step of the urea cycle, generating carbamoyl phosphate (CP)[129]. N-acetyl glutamate (NAG) is an essential activator of CPS1, and congenital NAGS mutations cause hyperammonemia[130]. CP is condensed with ornithine by ornithine carbamoyltransferase (OTC) to generate citrulline, which is exported through ORNT1 (the citrulline–ornithine antiporter for the remaining steps of the cycle). Interestingly, in *KRAS*/*LKB1*-mutant cancer, CP from CPS1 is diverted into de novo pyrimidine synthesis[131]. The mechanism of CP export from the mitochondria is unknown and may be a potential therapeutic target.

Although urea is a metabolic waste product for mammalian cells, urease-positive bacteria in the microbiome re-catabolize 15–30% of urea to regenerate ammonia[132]. Consequently, similar to congenital mutations in urea cycle enzymes, the microbiome can contribute to hyperammonemia[126,133]. Beyond ammonia metabolism, many microbial metabolites intersect host biology and their roles remain an active area of research[134].

GDH and GS assimilate ammonia, generating glutamate and glutamine. Glutamate contributes to the urea cycle through conversion to aspartate by GOT2 and mitochondrial export by AGC1/2. GDH is a bidirectional enzyme, and high ammonia levels reverse the direction of GDH, favouring its reductive activity[135]. This bi-directionality is particularly relevant in breast cancers, as ammonia accumulates in the TME, driving GDH towards glutamate synthesis[93]. Beyond the TME, physiological niches with high ammonia levels (the microbiome, liver and kidneys) may promote the reductive activity of GDH. Additionally, GDH-mediated ammonia assimilation requires NAD(P)H and therefore may contribute to redox balance.

ROS. Mitochondria generate, sequester and interconvert ROS in response to stressors such as hypoxia, nutrient availability, cytokine stimulation and changes in mitochondrial membrane potential[136]. ROS are generated from the reduction of oxygen (O_2) to superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). Mitochondrial ROS are generated in reactions such as NADPH oxidase (NOX4) and the Fenton reaction, and through electron leak from ETC complexes[123], although NOX4 is not strictly localized to mitochondria[137]. ROS are highly reactive and inflict oxidative damage to macromolecules[138].

Mitochondria rely on ROS clearance to protect the concentrated iron–sulfur clusters in the ETC and iron-dependent enzymes such as aconitase. Superoxide dismutase (SOD2) converts superoxide into a less reactive molecule, H_2O_2 (ref. [123]). Cellular H_2O_2 can be degraded to water by catalase, glutathione peroxidase (GPx) and peroxiredoxin (Prx), however, mitochondria do not have catalase and only a single splice variant of GPx4 has been demonstrated to localized in mitochondria[139,140]. Mitochondria rely on the combined activities of peroxiredoxins (Prx3 and Prx5), thioredoxins (Trx2) and thioredoxin reductase 2 (TRXR2) to decompose the locally generated H_2O_2 (ref. [141]).

Beyond toxicity, ROS are potent mitogen signalling agents that foster proliferation, differentiation and migration[123,142]. Specifically, ROS oxidize cysteine residues, linking mitochondria to signalling cascades. ROS inactivates the catalytic cysteine of phosphatase 1B (PTP1B), enabling receptor tyrosine phosphorylation required for growth-factor signaling[143]. ROS inactivate PTEN, which represses the PI-3 kinase–AKT signalling cascade and PHDs to repress HIF hydroxylation[144,145]. In breast cancer, low levels of the mitochondrial sirtuin 3 promote HIF stabilization through ROS, stimulating the Warburg effect[146]. In macrophages, mROS promote the antibacterial innate immune response, and mice harbouring mROS-deficient macrophages are susceptible to infection[147]. Similarly, mitochondria provide ROS for B cell and T cell activation[148,149]. ROS are thus critical to proliferating systems.

Hydrogen sulfide. Hydrogen sulfide (H_2S) is produced in the microbiome by sulfur-reducing bacteria and by mammalian cells through cystathionine-β synthase (CBS), cystathionine-γ lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST)[150]. H_2S-producing enzymes are localized to the cytosol and mitochondria, depending on the tissue type[124].

High levels of H_2S are toxic and repress respiration through complex IV inhibition[137]. To dampen H_2S toxicity, mitochondria sequentially oxidize H_2S, generating thiosulfate, sulfite and, ultimately, sulfate[151]. The first and last reactions, catalysed by flavin-dependent sulfide quinone reductase (SQR) and sulfite oxidase (SO), directly deposit electrons onto coenzyme Q (CoQ) in the ETC (ref. [136]). In CoQ deficiency, H_2S oxidation is significantly repressed[152]. The intermediate oxidation step of H_2S is catalysed by thiosulfate reductase (TR) and requires oxidized glutathione as an electron sink. Because the enzymes for glutathione synthesis are cytosolic, mitochondria must import glutathione for this process. Glutathione can utilize the dicarboxylate carrier SLC2510 and the α-KG carrier SLC25A11 for import, although a selective mechanism of transport remains unknown and may be pivotal for H_2S clearance[153].

H_2S metabolism is directly linked with oxidative phosphorylation[154]. Hypoxia represses H_2S detoxification through respiratory chain inhibition[124]. Interestingly, the microbiome, which has the highest H_2S levels, is hypoxic in some regions[155]. The mechanism for H_2S tolerance in the microbiome remains unknown. H_2S production and clearance may be critical in diseases such as cancer and diabetes, which are associated with altered respiration.

Future directions

Here, we discuss the multifaceted contributions of mitochondria to cell metabolism as bioenergetic powerhouses, biosynthetic centres, balancers of reducing equivalents and waste management hubs. Although mitochondrial pathways are well defined, the mechanisms by which metabolites are compartmentalized remain elusive. Identifying the transporters that coordinate metabolic flux for key pathways, such as amino acid and glutathione import, will be important directions for future research[156]. Given that mitochondrial metabolism is critical to many diseases, transporters that enable metabolic compartmentalization may be promising targets for therapeutics[5,65,157–159]. It will also be key to consider mitochondrial metabolite concentrations, which differ from whole cell concentrations[160], to better inform the kinetics of mitochondrial enzymes under different cellular stress conditions and in disease. Mitochondrial concentrations are critical when studying bi-directional enzymes, such as transaminases and enzymes in 1 C metabolism.

It will be important for future studies to probe the physiological contributions of mitochondria to cell biology. Metabolism is not always comparable when studying in vitro and in vivo models. These differences may dictate the efficacy of therapies, such as the glutaminase inhibitor in cancer[40]. The extent to which a physiological niche alters mitochondrial contributions to metabolism and cell/tissue function has not been well explored. For example, metabolic by-products accumulate in the TME, increasing the necessity

for cancer cells to engage waste management pathways[13,93,125]. Disparities between model systems may be avoided by performing in vitro studies in media with physiological metabolite concentrations, using model systems that represent the 3-dimensional architecture of the tissue being studied, and performing experiments in vivo[161–163]. Future studies in this exciting and growing field will continue to reveal the roles of mitochondrial metabolism in cellular homeostasis and disease.

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Competing interests

The authors declare no competing interests.

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