Mitochondrial energetics in the kidney

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Abstract | The kidney requires a large number of mitochondria to remove waste from the blood and regulate fluid and electrolyte balance. Mitochondria provide the energy to drive these important functions and can adapt to different metabolic conditions through a number of signalling pathways (for example, mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways) that activate the transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), and by balancing mitochondrial dynamics and energetics to maintain mitochondrial homeostasis. Mitochondrial dysfunction leads to a decrease in ATP production, alterations in cellular functions and structure, and the loss of renal function. Persistent mitochondrial dysfunction has a role in the early stages and progression of renal diseases, such as acute kidney injury (AKI) and diabetic nephropathy, as it disrupts mitochondrial homeostasis and thus normal kidney function. Improving mitochondrial homeostasis and function has the potential to restore renal function, and administering compounds that stimulate mitochondrial biogenesis can restore mitochondrial and renal function in mouse models of AKI and diabetes mellitus. Furthermore, inhibiting the fission protein dynamin 1-like protein (DRP1) might ameliorate ischaemic renal injury by blocking mitochondrial fission.

The kidney is one of the most energy-demanding organs in the human body. A study measuring the resting energy expenditure of various organs in healthy adults, ranging from 21 to 73 years of age, found that the kidney and heart have the highest resting metabolic rates[1]. The kidney has the second highest mitochondrial content and oxygen consumption after the heart[2,3]. The resting metabolic rate for the kidney is high because the kidney requires an abundance of mitochondria to provide sufficient energy to enable it to remove waste from the blood, reabsorb nutrients, regulate the balance of electrolytes and fluid, maintain acid–base homeostasis, and regulate blood pressure. These tasks, especially the reabsorption of glucose, ions and nutrients through channels and transporters, are driven by ion gradients.

Mitochondria provide energy to the Na^+–K^+-ATPase to generate ion gradients across the cellular membrane[4]. In the kidney, the proximal tubule, the loop of Henle, the distal tubule and the collecting duct all require active transport to reabsorb ions[4]. By contrast, glomerular filtration is a passive process that is dependent on the maintainence of hydrostatic pressure in the glomeruli[5]. Proximal tubules require more active transport mechanisms than other renal cell types because they reabsorb 80% of the filtrate that passes through the glomerulus, including glucose, ions, and nutrients. As such, they contain more mitochondria than any other structure in the kidney. The ability of mitochondria to sense and respond to changes in nutrient availability and energy demand by maintaining mitochondrial homeostasis is critical to the proper functioning of the proximal tubule. In this Review, we describe the processes involved in maintaining mitochondrial homeostasis and discuss how these processes provide and maintain sufficient energy to support renal function. We also explore how disease states, such as acute kidney injury (AKI) and diabetic nephropathy, alter mitochondrial function, and how mitochondrial energetics might be targeted as a treatment for these diseases.

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Mitochondrial function

Mitochondria are a network of plastic organelles that together maintain a variety of cellular functions and processes, such as the level of reactive oxygen species (ROS), cytosolic calcium and apoptosis[6]. Most importantly, mitochondria produce ATP, thereby supplying the energy source for basal cell functions as well as cellular repair and regeneration. To accomplish this feat, a population of healthy and functional mitochondria is vital.

ATP production

Aerobic respiration involves the consumption of oxygen to produce ATP, water and carbon dioxide (CO_2). Most of the ATP generated by aerobic respiration is produced by the flux of electrons through the electron transport chain (ETC) in a process called oxidative phosphorylation (FIG. 1a). Aerobic respiration begins with the production of pyruvate from glucose via glycolysis[7]. Pyruvate is

Key points

- Mitochondrial homeostasis requires a fine-tuned balance between mitochondrial dynamics and mitochondrial energetics, and ensures the maintenance of properly functioning mitochondria
- Mitochondria can adapt to different metabolic conditions via the regulation of mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) nutrient sensing pathways, to maintain a healthy population of mitochondria
- External stimuli can augment mitochondrial processes, such as mitophagy, fission and fusion, and mitochondrial biogenesis to attenuate irregular levels of ATP production
- The disruption of mitochondrial homeostasis in the early stages of acute kidney injury is an important factor that drives tubular injury and persistent renal dysfunction
- Hyperglycaemia-induced ATP depletion triggers changes in mitochondrial morphology that lead to the onset of diabetic nephropathy in diabetes mellitus
- Correcting abnormal electron transport chain function directly, and/or by targeting the pathways that regulate mitochondrial biogenesis, is likely to improve renal outcomes by restoring mitochondrial function and stimulating organ repair

converted to acetyl-CoA (via pyruvate dehydrogenase in the mitochondrial matrix), which fuels the tricarboxylic acid (TCA) cycle to produce six NADH, four $FADH_2$, and six CO_2 per molecule of glucose[7]. Electrons from NADH and $FADH_2$ are transferred to complex I and complex II, respectively, of the ETC in the mitochondrial inner membrane. Electrons then travel through the ETC to complex IV, where they are accepted by oxygen. Note that the haem protein cytochrome *c*, which is located in the mitochondrial inner membrane, facilitates the transfer of electrons from complex III to complex IV. Ultimately, protons, which are actively pumped into the intermembrane space as electrons move through complexes I, III, and IV, flow through ATP synthase (also known as complex V) to drive the conversion of ADP to ATP[7].

In general, all cell types in the kidney need ATP to maintain cellular functions; however, the mechanism by which ATP is produced is cell type-dependent. For example, in the renal cortex, proximal tubules depend on the efficiency of oxidative phosphorylation to produce ATP that drives the active transport of glucose, ions and nutrients[8]. By contrast, glomerular cells, including podocytes, endothelial cells and mesengial cells, have lower oxidative capacity because their function is to filter blood to remove small molecules (namely, glucose, urea, water and salts) while retaining large proteins, such as haemoglobin[9]. This passive process does not directly require ATP and, therefore, glomerular cells have the ability to perform aerobic and anaerobic respiration to produce ATP for basal cell processes[10–13]. Anaerobic respiration, like aerobic respiration, begins with glycolysis, producing pyruvate from glucose, but is characterized by the subsequent production of lactate from pyruvate[14]. Anaerobic respiration produces two molecules of ATP and is an efficient mechanism for cell types that have a lower O_2 supply[10]. This process is important, as glycolysis frequently occurs in cell types other than proximal tubules and can utilize alternative energy sources, such as amino acids, in the absence of glucose[15,16]. For example, pyruvate can be generated via the oxidation of amino acids to fuel both anaerobic and aerobic mechanisms of ATP production.

Carnitine shuttle
Enzymes in the mitochondrial membrane that transport long-chain fatty acids from the cytosol to the mitochondrial matrix by replacing their coA group with carnitine.

Due to the high energy demand of proximal tubules, aerobic respiration is their primary mechanism of ATP production. Proximal tubules utilize non-esterified fatty acids, such as palmitate, via β-oxidation for maximal ATP production. A single molecule of palmitate produces 106 molecules of ATP, whereas the oxidation of glucose only yields 36 molecules of ATP[17,18]. Fatty acids are taken up by proximal tubule cells via transport proteins, such as platelet glycoprotein 4 (also known as CD36), or synthesized in the cytoplasm, where they are activated by coA before being transported into mitochondria through the carnitine shuttle[19] (FIG. 1b). Specifically, carnitine *O*-palmitoyltransferase 1 (CPT1) exchanges the coA group on fatty acids with L-carnitine, allowing the transfer of fatty acids across the mitochondrial inner membrane space through the carnitine shuttle. Fatty acids are then broken down for energy via β-oxidation in the mitochondrial matrix. Although β-oxidation is the most efficient mechanism for producing ATP in proximal tubules, it is important to note that due to the high consumption of oxygen by proximal tubules, they are more susceptible than other cell types to changes in oxygen levels[20,21]. A decrease in oxygen levels can lead to impaired β-oxidation and a reduction in ATP production (see below).

A balance of catabolic and anabolic nutrient-sensing pathways regulates the optimum concentration of fatty acids in a cell (see below). Disease states and different metabolic conditions in the kidney alter this balance and can adversely affect mitochondrial energetics. For example, the accumulation of fatty acids in AKI and diabetic nephropathy can negatively impact ATP production by decreasing β-oxidation in the mitochondria and increasing the formation of lipid droplets inside the cell[18]. An inverse correlation exists between lipogenesis that is induced by the accumulation of fatty acids and the transcription of genes that are involved in fatty acid oxidation[22,23]. Fatty acids can also trigger apoptosis and, more importantly, create a toxic environment inside the cell that hinders mitochondrial function[24,25]. Fatty acid metabolism in disease states, such as AKI and diabetic nephropathy, will be discussed below.

Antioxidant defences

As discussed, mitochondria produce ATP via the ETC. At steady state, when electrons are passed through the ETC to molecular oxygen, a low concentration of superoxide anions is generated from complex I and complex III. Although a low level of ROS, such as superoxide anions, is important for cell function, high concentrations are toxic to mitochondria and the cell[26–28] (FIG. 2). For example, under oxidative stress, increased levels of ROS can cause breaks in mitochondrial DNA (mtDNA) that cause mutations in the next generation of mitochondria; breaks in mtDNA also negatively affect the efficiency of the ETC, causing a decrease in ATP production and damaging proteins and lipids[29]. ROS can also trigger apoptosis in the cell by causing the release of cytochrome *c*, leading to mitochondrial dysfunction[29]. Therefore, mitochondria have antioxidant defence systems to counteract the excessive formation of additional

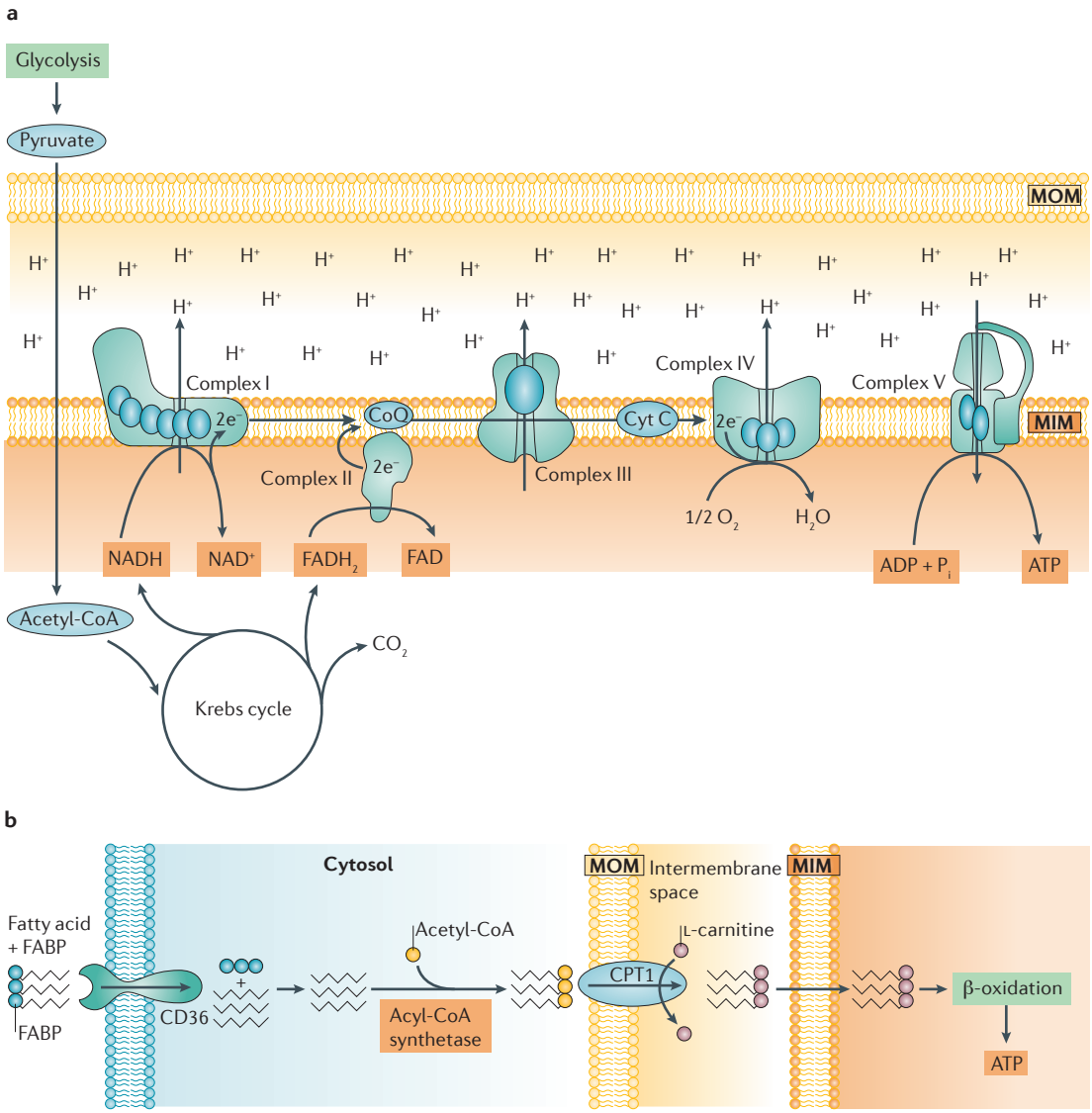

Figure 1 | **ATP production in the kidney. a** | The electron transport chain (ETC). A functioning ETC transforms reducing equivalents from NADH and $FADH_2$ to produce NAD^+ and FAD^+, respectively. The electrons (e^-) that are produced travel through the complexes of the ETC and are ultimately accepted by oxygen at complex IV. As electrons are transferred from complex to complex, protons (H^+) are actively pumped out from complexes I, III, and IV into the intermembrane space, maintaining the membrane potential and driving the production of ATP by ATP synthase (also known as complex V). **b** | Fatty acid transport and activation in renal proximal tubule cells. Proximal tubules require large amounts of ATP to drive ion transport and therefore rely on aerobic respiration, the most efficient mechanism for producing ATP. Fatty acids are a main source of energy for proximal tubules because more ATP can be produced from one molecule of palmitate than from one molecule of glucose[18]. Fatty acids bound to fatty acid-binding proteins (FABP) are transported into the proximal tubule cell via platelet glycoprotein 4 (also known as CD36) and activated by the addition of acetyl-CoA in the cytosol via acyl-CoA synthetase. Activated fatty acids are transported into mitochondria via carnitine *O*-palmitoyltransferase 1 (CPT1), which exchanges their acyl-CoA group for L-carnitine, whereupon they undergo β-oxidation to produce ATP. CoQ, coenzyme Q; Cyt C, cytochrome *c*; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane; P_i, inorganic phosphate.

ROS. Superoxide dismutase 2 (SOD2), which converts superoxide anions to hydrogen peroxide and oxygen, is specific for mitochondria[30]. Moreover, the transcription of genes encoding antioxidant enzymes, such as SOD2, catalase and glutathione peroxidase, is activated by nuclear factor erythroid 2-related factor 2 (NRF2) in response to oxidative stress, providing a mechanism to prevent excessive ROS production[31]. The importance of these antioxidant systems is to maintain optimal ATP production and sustain mitochondrial function.

Another important antioxidant defence mechanism involves glutathione. Glutathione is a tripeptide (γ-glutamyl-cysteinal-glycine) nucleophile that can exist in a reduced form (GSH), or in an oxidized form

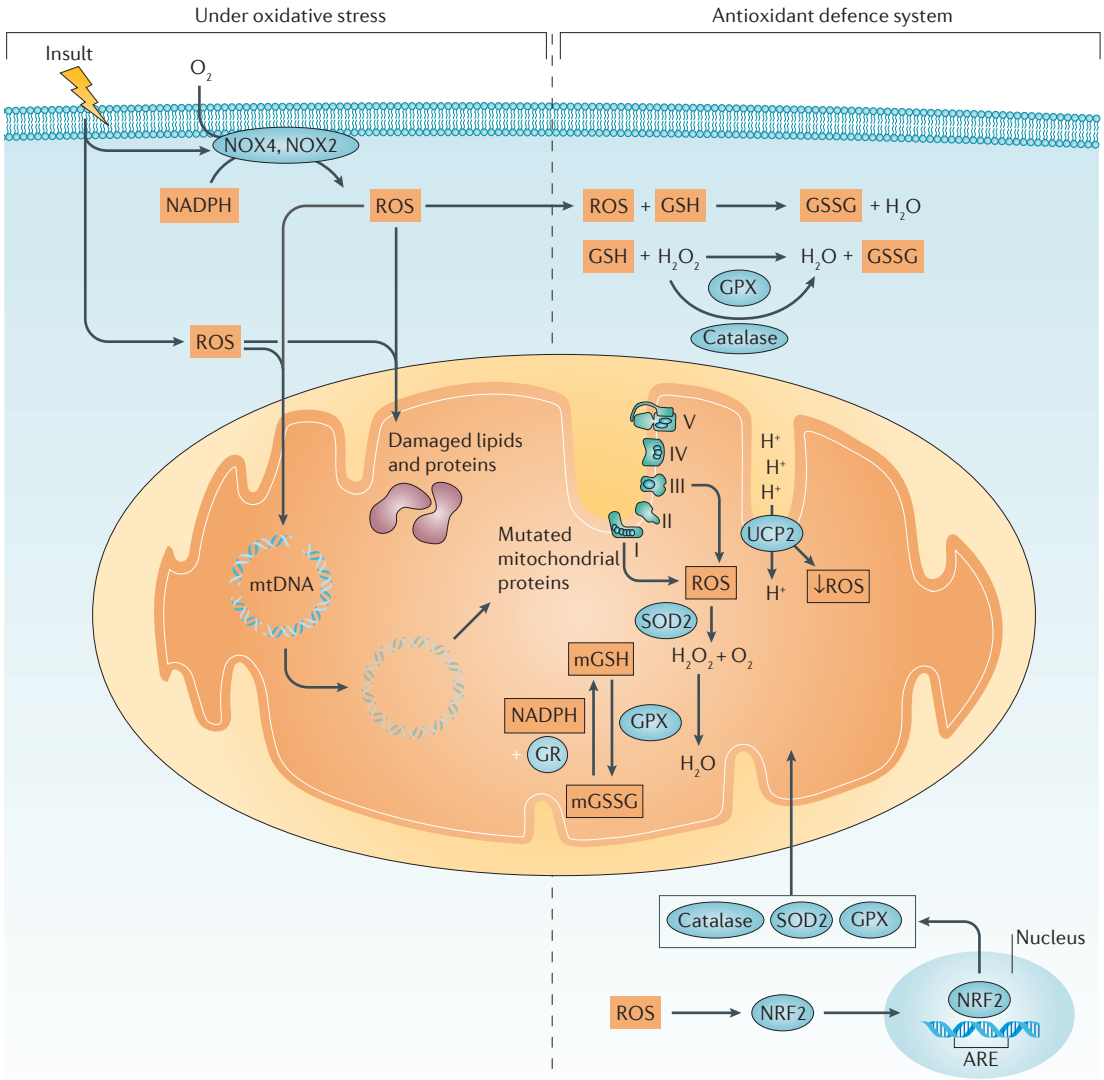

Figure 2 | **Oxidative stress and the antioxidant defence system.** Insults can increase the production of reactive oxygen species (ROS) in the cytosol and mitochondria. NADPH oxidase 2 (NOX2) and NOX4 can also contribute to the production of ROS[222]. The production of ROS can cause breaks in mitochondrial DNA (mtDNA) and damage lipids and proteins. Damaged mtDNA can produce aberrant mitochondrial proteins and prevent mitochondrial protein synthesis, whereas damaged lipids and proteins result in impaired mitochondrial function, leading to further increases in mitochondrial ROS. ROS also activate nuclear factor erythroid 2-related factor 2 (NRF2), which translocates to the nucleus and binds to antioxidant-responsive elements (AREs) to activate the transcription of genes encoding oxidant-neutralizing enzymes, such as mitochondrial superoxide dismutase 2 (SOD2), glutathione peroxidase (GPX) and catalase. SOD2 reduces superoxide anions to hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase, found in the cytoplasm, and GPX, located in the cytoplasm and mitochondria, reduce H_2O_2 to water (H_2O)[223]. GPX also oxidizes glutathione (GSH), resulting in glutathione disulfide (GSSG) as a byproduct of reducing hydrogen peroxide to water. GSSG in mitochondria (mGSSG) is converted back to GSH by glutathione reductase (GR) in a process that requires the presence of NADPH. The activity of the mitochondrial uncoupling protein 2 (UCP2) is increased, dissipating the proton motive force and decreasing ROS production. mGSH, mitochondrial GSH. The electron transport chain complexes I–V are indicated as I, II, III, IV and V.

as glutathione disulfide (GSSG). Mitochondria contain their own pool of glutathione, mitochondrial glutathione (mGSH), which not only helps to decrease excessive ROS levels but also prevents the release of cytochrome *c* from the inner membrane[32]. mGSH directly interacts with superoxide anions and becomes oxidized to GSSG[33]. Glutathione peroxidase (GPX) is located in both the cytoplasm and the mitochondria and uses GSH to reduce hydrogen peroxide to water, resulting in GSSG as a by-product[34]. GSSG cannot exit the mitochondria and is converted back to mGSH by glutathione reductase, for reuse or for elimination from the mitochondria[33]. The conversion of GSSG to mGSH requires NADPH, allowing crosstalk between the mechanism that maintains mGSH levels and the pentose phosphate pathway that produces NADPH. Together, these mechanisms have a major role in preventing excessive levels of ROS, and sustaining mitochondrial function.

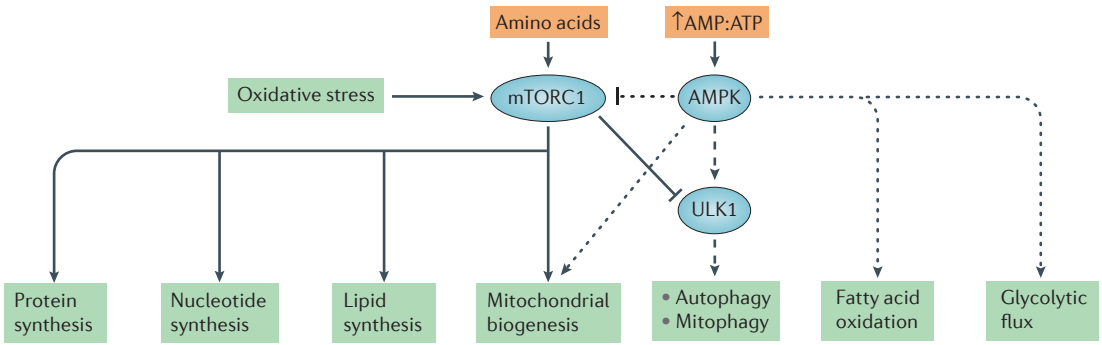

Figure 3 | **Crosstalk between two nutrient-sensing pathways.** Mechanistic target of rapamycin complex 1 (mTORC1) and AMP-activated protein kinase (AMPK) have key roles in regulating mitochondrial biogenesis and mitophagy. mTORC1 is responsible for triggering anabolic pathways, such as the synthesis of proteins, nucleotides and lipids, as well as mitochondrial biogenesis. AMPK activates catabolic pathways, including autophagy, mitophagy, fatty acid oxidation and glycolysis. AMPK can stimulate mitochondrial biogenesis (dotted arrow). However, in response to stimuli such as nutrient deprivation, AMPK can inhibit mTORC1 (dotted inhibitory line) and phosphorylate ULK1 to activate mitophagy (dashed arrow). Together these two signalling pathways maintain cell function and sustain mitochondrial energetics in response to stimuli such as hypoxia, oxidative stress and energy depletion.

Uncoupling proteins are a family of mitochondrial transport proteins that are located in the mitochondrial inner membrane[35,36]. They transport protons across the inner membrane to the mitochondrial matrix. Mitochondrial uncoupling protein 2 (UCP2) is expressed in the kidney and is activated by mitochondrial ROS and other stimuli. An increase in ROS formation in the mitochondria activates UCP2, dissipating the proton motive force as heat and, as a result, reducing ROS production[36,37]. As ROS production contributes to mitochondrial dysfunction in AKI and diabetic nephropathy, UCP2 has been explored in the kidney and in these disease states[38]. Studies investigating the role of *UCP2* polymorphisms in the kidney that exacerbate disease in patients with diabetic nephropathy reveal that UCP2 is a potential target for treatment[39]. Lack of UCP2 has also been shown to worsen tubular injury after induction of experimental AKI in mice[38]. These studies show the importance of UCP2 in the kidney as well as its role in attenuating excessive ROS production.

Mechanisms also exist to sustain mitochondrial function under hypoxic conditions. The lack of oxygen under hypoxic conditions decreases ATP production and causes cell death. In normoxic conditions, hypoxia-inducible factor 1α (HIF1α) is degraded in the presence of oxygen and α-ketoglutarate, an intermediate of the TCA cycle[40]. However, under hypoxic conditions, HIF1α heterodimerizes with HIF1β to form a transcription factor that binds to a hypoxia response element (HRE) present in genes that encode glycolytic enzymes and glucose transporters in the kidney[41]. Hypoxic conditions also alter the composition of complex IV of the ETC in which, at physiological conditions, the regulatory subunit 1 predominates in the ETC; during hypoxia, regulatory subunit 2 predominates in complex IV, which increases the efficiency of the ETC[42]. Several studies have shown that increasing the efficiency of the ETC increases the production of mitochondrial ROS under hypoxic conditions, although the mechanism by which this occurs is still unclear[43–45]. The effects of oxidative stress and hypoxia on mitochondrial morphology and energetics are discussed below.

Nutrient-sensing pathways in the kidney

Nutrient-sensing pathways can directly affect mitochondrial energetics in response to external stimuli, such as hypoxia, oxidative stress and energy depletion. Two signalling pathways in particular have been extensively explored in the kidney, namely the mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) signalling pathways[46,47]. Both signalling pathways also have a role in regulating mitochondrial biogenesis — that is, the production of new and functional mitochondria — to help maintain a healthy population of mitochondria (FIG. 3).

mTOR is a serine/threonine kinase complex that comprises a number of proteins. Two distinct mTOR complexes exist: mTOR complex 1 (mTORC1) and mTORC2, each of which contain their own unique subunits and substrates. mTORC1, which is a complex of mTOR, regulatory-associated protein of mTOR (Raptor) and several other proteins, regulates cell growth and proliferation and inhibits autophagy by stimulating anabolic processes. mTORC2, which is a complex of mTOR, rapamycin-insensitive companion of mTOR (Rictor) and several other proteins, is thought to regulate potassium and sodium levels in the kidney[48,49]. mTORC1 is considered a nutrient sensor because it can be activated by growth factors, nutrients such as amino acids and glucose, and oxidative stress, triggering pathways that lead to protein synthesis, nucleotide synthesis, lipid synthesis and mitochondrial biogenesis by activating the transcriptional repressor yin and yang 1 (YY1)[46,50]. In the case of mitochondrial biogenesis, YY1 acts as a transcription factor and co-activator of the master regulator of mitochondrial biogenesis — the transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) — resulting in the transcription of mitochondrial genes[50]. mTORC1-deficiency specifically in renal

proximal tubules of mice decreased the protein levels of PGC1α *in vivo*[51]. Of note, the mTOR pathway can be inhibited by hypoxia and AMPK.

AMPK is another nutrient sensor in the kidney that stimulates catabolic processes. When the AMP:ATP ratio in the cell is high in the presence of low oxygen levels, AMPK is activated[52]. AMPK targets a number of proteins, the phosphorylation of which leads to the production of antioxidant enzymes, the induction of mitochondrial biogenesis, and an increase in glycolytic flux, fatty acid oxidation and glucose transport; all of these events contribute to cell growth and an increase in cellular metabolism[53]. AMPK can induce mitochondrial biogenesis by stimulating the transcription of the gene encoding PGC1α (*PPARGC1A*) and by phosphorylating PGC1α at Thr177 and Ser539 to increase its activity[54]. AMPK stimulates the production of energy and inhibits energy-consuming pathways by inhibiting mTORC1. Under conditions of nutrient deprivation, crosstalk exists between mTORC1 and AMPK (FIG. 3) so that AMPK can inhibit mTORC1 while activating autophagy by phosphorylating the serine/threonine protein kinase ULK1 (REF. 55). Due to the presence of AMPK targets in kidney cells, AMPK is a novel drug target for several renal diseases (see below).

Maintaining mitochondrial homeostasis

Mitochondrial homeostasis requires a balance between mitochondrial biogenesis, fission and fusion, and mitophagy — the selective removal of non-functional and damaged mitochondria from cells by autophagy. All of these processes work together to maintain mitochondrial energetics, that is, the optimal production of ATP in normoxic conditions and in altered metabolic conditions.

Mitochondrial biogenesis

Mitochondrial biogenesis, which produces new and functional mitochondria, increases ATP production in response to increasing energy demands. Mitochondrial biogenesis is regulated by a range of transcriptional co-activators and co-repressors[56,57]. One study has shown that PGC1α is a prominent regulator, at the transcriptional level, of oxidative phosphorylation, the TCA cycle and fatty acid metabolism in the kidney[58]. In that study, the investigators performed gene expression profiling of kidneys from control mice and nephron-specific inducible *PPARGC1A*-knockout (NiPKO) mice that had been fed a chow diet or high-fat diet (HFD). Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, they analysed transcripts from all four groups of mice, and found a decrease in transcripts related to oxidative phosphorylation, TCA cycle and glycolysis in chow-fed NiPKO mice and in HFD-fed NiPKO mice. This finding supports the idea that inactivation of PGC1α in the kidney reduces mitochondrial function and metabolism and subsequently decreases mitochondrial biogenesis.

Overexpression of PGC1α can also mitigate mitochondrial dysfunction *in vitro* after oxidant exposure, further supporting a role for mitochondrial biogenesis in mitochondrial homeostasis[59]. The activation of peroxisome proliferator-activated receptors (PPARs) and oestrogen-related receptors (ERRs) also contributes to the regulation of mitochondrial biogenesis, sometimes by these receptors directly interacting with PGC1α[60] (FIG. 4). PPARs and ERRs are nuclear receptors that can be activated by fatty acids and steroid hormones such as oestrogen, and they elicit a response by binding to specific DNA response elements through their DNA-binding domains[61]. PGC1α can directly bind to these nuclear receptors and co-activate the transcription of genes, the protein products of which are involved in oxidative phosphorylation and fatty acid oxidation[62,63]. PGC1α activation results in its translocation from the cytoplasm to the nucleus, allowing it to upregulate the transcription of genes that are important for mitochondrial homeostasis and ATP production[64]. Transcription programmes downstream of PGC1α include nuclear and mitochondrial genes, as well as those involved in signalling pathways that regulate mitochondrial biogenesis (reviewed elsewhere[65–67]).

As the activation or suppression of PGC1α is regulated by external stimuli and post-translational modifications, it can be considered to be a nutrient sensor in the kidney. The expression and regulation of PGC1α in the kidney is still being explored. However, much of what is known about the regulation of PGC1α was discovered in the injured kidney as a result of disease states, such as diabetic nephropathy, ischaemia–reperfusion injury (IRI), sepsis, and cisplatin-induced AKI. Findings in these disease states support a role for PGC1α in the recovery phase from these diseases and in restoring mitochondrial function, highlighting PGC1α as a therapeutic target. Exercise and insulin stimulate an increase in *PPARGC1A* expression in skeletal muscle and in the heart, whereas fasting increases *PPARGC1A* expression in the liver[65,68]. In brown fat and muscle cells, cold exposure activates PGC1α[65]. In cases of oxidative stress or nutrient depletion, the activation of mitochondrial biogenesis helps rescue mitochondria from apoptosis[69,70]. In general, if the cell is in need of more energy, PGC1α is activated by deacetylation, whereas PGC1α is inactivated by acetylation when energy levels are high[65].

In addition to AMPK and mTOR, other energy sensing pathways that stimulate mitochondrial biogenesis include those involving sirtuins, cAMP and cyclic guanosine monophosphate (cGMP) (FIG. 4). Sirtuin 1 (SIRT1) and SIRT3 are protein deacetylases that have a role in a variety of mitochondrial processes, including the ETC, TCA cycle, fatty acid oxidation, redox homeostasis and mitochondrial biogenesis[71]. SIRT1 activity is activated by NAD^+, after which it activates downstream targets such as PGC1α[64]. SIRT3 is mitochondria-specific and can be activated to stimulate mitochondrial biogenesis[72]. The stimulation of adenylyl cyclase results in an increase in cAMP, which activates protein kinase A (PKA) that in turn phosphorylates cyclic AMP-responsive element-binding protein (CREB)[65,73]. CREB is also a transcriptional activator of PGC1α and can therefore also stimulate mitochondrial biogenesis[73]. Finally, increased levels of cGMP induced by caloric

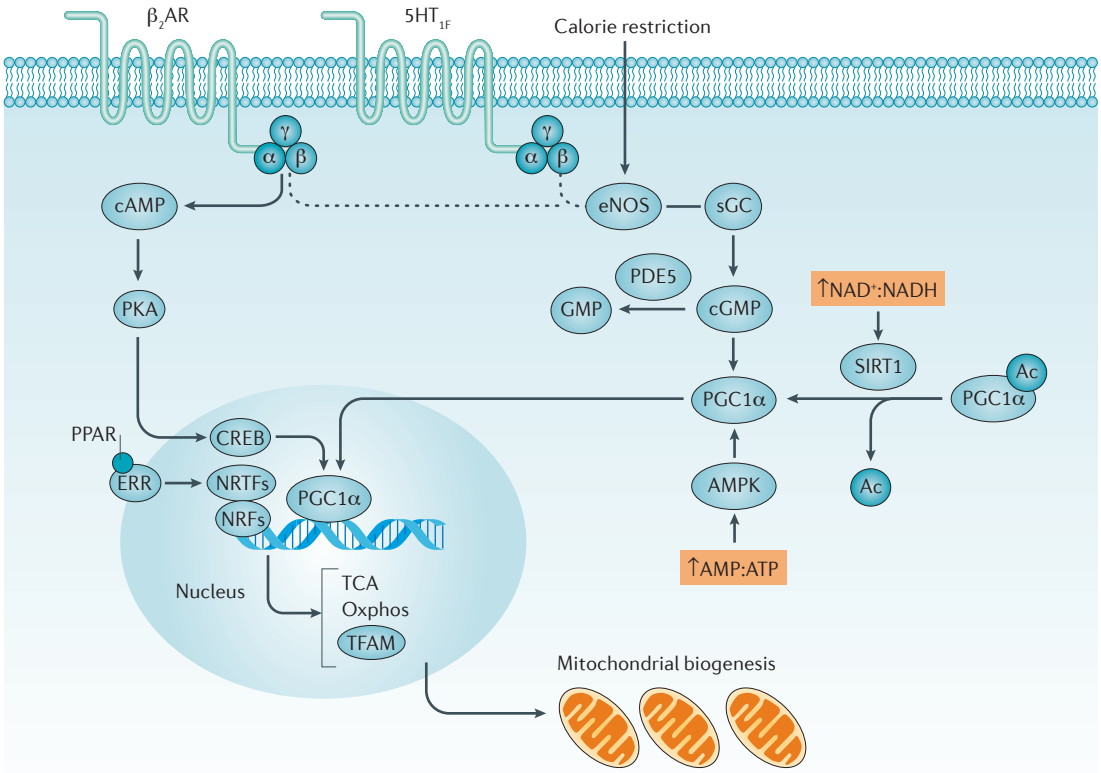

Figure 4 | **Activation and regulation of mitochondrial biogenesis.** A complex network of pathways regulate mitochondrial biogenesis. Activation of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) in the cytosol causes its translocation to the nucleus and the transcription of genes (including that encoding mitochondrial transcription factor A (TFAM)), the protein products of which are needed for oxidative phosphorylation (oxphos), the tricarboxylic acid (TCA) cycle and mitochondrial biogenesis. TFAM aids in the transcription of genes that are encoded by mitochondrial DNA[224–226]. The activation of G protein-coupled receptors (GPCRs), such as the β_2 adrenergic receptors (β_2AR) and 5-hydroxytryptamine receptor 1F (5-HT_{1F}), leads to the dissociation of heterotrimeric G proteins composed of Gα, Gβ and Gγ subunits and the subsequent activation of protein kinase A and endothelial nitric oxide synthase (eNOS)[66]. The pathway from GPCRs to eNOS is still under investigation, as indicated by the dashed line. eNOS stimulates soluble guanylyl cyclase (sGC) to form cyclic guanosine monophosphate (cGMP), which in turn activates PGC1α. A number of compounds can activate nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) and oestrogen-related receptors (ERRs) and induce mitochondrial biogenesis. Once activated, these nuclear receptors can act as transcriptional co-activators (labelled in the figure as nuclear receptor transcription factors (NRTFs)), with PGC1α to stimulate mitochondrial biogenesis. Other transcription factors, including nuclear respiratory factor 1 (NRF1) and NRF2, can also directly bind to PGC1α to induce mitochondrial biogenesis[227]. Stimuli, such as caloric restriction, can activate eNOS, increasing the production of cGMP and leading to the activation of PGC1α. The activity of sirtuin 1 (SIRT1) is increased in the presence of a high ratio of NAD^+ to NADH concentrations, leading to the activation of PGC1α. High AMP:ATP ratios also activate AMP-activated protein kinase (AMPK), activating PGC1α by phosphorylation. In all of these cases, the activation of PGC1α stimulates mitochondrial biogenesis. Ac, acetyl; PDE5, cGMP-specific 3′,5′-cyclic phosphodiesterase; PKA, protein kinase A; sGC, soluble guanylyl cyclase.

restriction and the inhibition of phosphodiesterases can stimulate PGC1α activation and mitochondrial biogenesis *in vivo*[74–76]. Several of these pathways are being targeted to increase mitochondrial biogenesis to correct mitochondrial defects.

Mitochondrial dynamics and energetics

Correct mitochondrial morphology must be maintained for maximal ATP production. The processes of fission, fusion and mitophagy drive mitochondrial dynamics as they directly affect mitochondrial structure and morphology. Fission and fusion complement each other under different metabolic conditions to maintain mitochondrial morphology, whereas mitophagy removes damaged mitochondria from the network[77]. Sustaining mitochondrial dynamics is important for the appropriate maintenance of mitochondrial energetics.

Fission and fusion. Fission, the splitting of mitochondria into two, and fusion, the combining of two mitochondria, are complementary processes that are necessary for mitochondrial homeostasis. At steady state there is a balance between these processes (FIG. 5). The genetic deletion of genes, the protein products of which are involved in fission or fusion, causes human disease. For example, dominant optic atrophy is characterized by a loss of visual acuity owing to mutations in the gene encoding the fusion protein dynamin-like 120 kDa protein (also

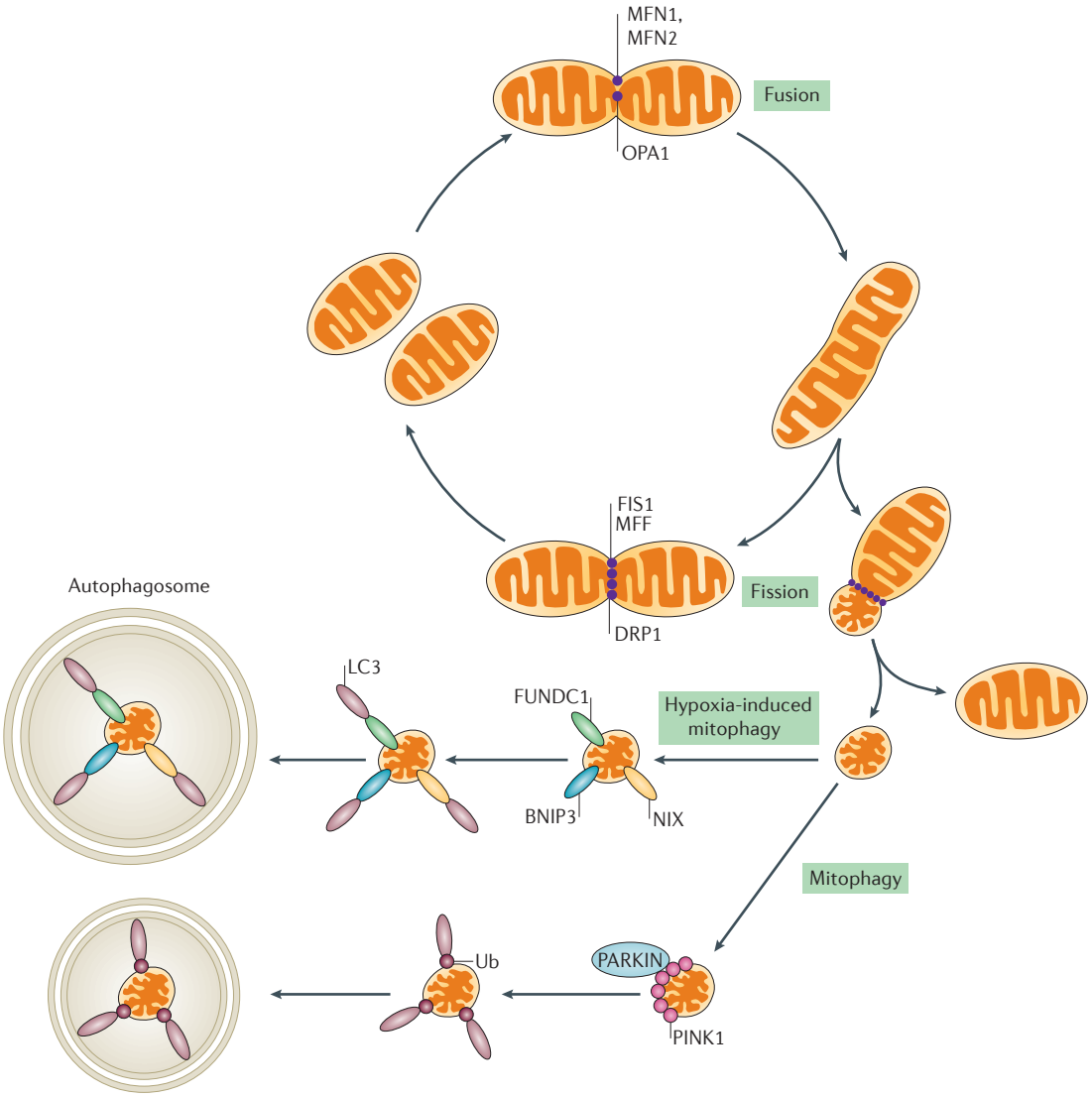

Figure 5 | **Mitochondrial dynamics: fission, fusion and mitophagy.** Mitochondria are dynamic organelles that need to maintain their morphology for the optimal production of ATP under different metabolic conditions and as part of a healthy network of mitochondria. Fission and fusion are two processes that are necessary for the maintenance of mitochondria morphology. Mitochondria fuse together via mitofusin 1 (MFN1) and MFN2 (outer membrane fusion) and the activation of dynamin-like 120 kDa (OPA1) (inner membrane fusion). Fusion can occur to maintain ATP production or to redistribute mitochondrial proteins. Fission can isolate depolarized mitochondrion that might not contribute to the healthy network of mitochondria. The activation of fission causes the oligomerization of dynamin 1-like protein (DRP1) on the mitochondrial outer membrane, where it is bound to receptors (namely mitochondrial fission 1 (FIS1) and mitochondrial fission factor (MFF)), forming a ring-like structure that mediates the separation of mitochondria. The network also isolates dysfunctional mitochondria for degradation by mitophagy via a well-studied PTEN-induced putative kinase 1 (PINK1)–PARKIN mechanism. Under adverse conditions such as hypoxia, however, mitochondria will be removed by a FUN14 domain-containing protein 1 (FUNDC1) or BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein (NIX)-dependent mechanism of mitophagy. LC3, microtubule-associated protein 1 light chain 3; Ub, ubiquitin.

known as OPA1), and mutations in the gene encoding the fission protein dynamin 1-like protein (DRP1), are lethal[78–83]. Although exceptions exist, in general, studies have shown that oxidative phosphorylation increases with fusion and decreases with fission to match the energy demands of the cells[84,85]. Excessive fusion, like excessive fission, can be associated with disease states, as seen in neurodegenerative diseases[86]. However, some cell types do not adhere to this trend, such as adult cardiomyocytes and senescent cells. Mitochondria in adult cardiomyocytes have a fragmented morphology but maintain oxidative capacity, whereas mitochondria in senescent cells remain elongated, which is characteristic of increased fusion[87]. Senescent cells in this elongated state have decreased bioenergetic capacity[88,89].

Fusion is a two-part process that involves fusion of the outer mitochondrial membrane and, subsequently, the inner mitochondrial membrane of two

mitochondria. GTPases of the dynamin superfamily — mitofusin 1 (MFN1), MFN2 and OPA1 — are key players in fusion. MFN1 and MFN2 are located on the outer mitochondrial membrane and are necessary for outer membrane fusion, whereas OPA1 resides in the inner membrane and is important for inner membrane fusion. Fusion leads to the elongation of mitochondria under physiological conditions, which can help to maintain oxidative phosphorylation[90]. These GTPases have a role in mitochondrial energetics. For example, deletion of *MFN2* in mice causes deficiency in coenzyme Q, an electron carrier in complex III, which leads to ETC dysfunction and a decrease in ATP production[91]. Activation of these mitofusins and the cleavage of OPA1 can be regulated by changes in metabolism (see below).

Mitochondrial outer membranes are tethered by dimerization of MFN1 and MFN2, and external stimuli, such as oxidative stress, can enhance outer membrane fusion[92]. The activation of inner membrane fusion can be regulated by changes in metabolism at sites of proteolytic cleavage of OPA1 (REF. 93). OPA1 usually exists in a soluble long form and can be cleaved by the ATP-dependent zinc metalloproteinase YME1L or by the metalloendopeptidase OMA1, which is activated in response to a loss in membrane potential, to yield a soluble short form[85]. The soluble long and soluble short forms of OPA1 are necessary for fusion to occur. During steady state, both forms can coexist to induce minor structural remodelling of mitochondria[94,95]. The activation of cleaved OPA1 requires the presence of GTP, and the availability of GTP to activate OPA1 correlates with ATP levels in the cell[96,97]. The exact mechanism by which outer membrane and inner membrane fusion events are coordinated is still under investigation.

Fission is necessary to isolate damaged mitochondria from the mitochondrial network. If the resulting daughter mitochondria are unbalanced and depolarized, they are targeted for mitophagy[98] to sustain a population of healthy mitochondria. However, excessive fission, as seen in diseases such as diabetic nephropathy and AKI, can have harmful effects on mitochondrial homeostasis in the long term[99]. *In vitro* studies to elucidate the mechanisms that trigger mitochondrial fission have shown that cells that are exposed to an excess of nutrients or oxidative stress have fragmented mitochondria[99]. Fission is induced by the translocation of DRP1 from the cytosol to the mitochondrial outer membrane as a result of a loss in mitochondrial membrane potential. If the membrane potential is not restored, mitochondria are degraded via mitophagy[99]. DRP1 oligomerizes on the outer membrane to form a ring-like structure around the mitochondria, which can cause scission of the membrane[100]. DRP1 can bind to several different receptors, such as mitochondrial fission 1 (FIS1), the mitochondrial dynamics proteins MID49 and MID51, and mitochondrial fission factor (MFF), which reside on the mitochondrial outer membrane[81]. DRP1 accumulates on the outer mitochondrial membrane by binding to these receptors and mediates the scission of mitochondria, which is dependent on GTP[101]. MID51 contains a cytosolic domain that has affinity for ADP and GDP, and can therefore act as a metabolic sensor[102,103]. DRP1 activity can be regulated by post-translational modifications, such as phosphorylation, ubiquitylation, and sumoylation[104], and several signalling pathways have been shown to regulate the phosphorylation of DRP1 (REF. 105). For example, phosphorylation of DRP1 at Ser637 by PKA inhibits its GTPase activity and thus inactivates fission[81,106]. By contrast, dephosphorylation of DRP1 at Ser637 by calcium and calmodulin-dependent serine/threonine protein phosphatase 2B catalytic subunit α isoform or calcineurin (CaN) activates DRP1 and promotes fission[107,108]. The balance between fission and fusion to maintain a functional population of mitochondria is an intricate process and is still under investigation. Mitochondria that disrupt this balance between fission and fusion, such as damaged mitochondria, are however removed from the network via mitophagy.

Mitophagy. Mitophagy in most cell types is regulated by a PTEN-induced putative kinase 1 (PINK1)–PARKIN mechanism that tags mitochondria for degradation[109]. PINK1, a kinase that is located in the cytosol, is imported into the mitochondria and then degraded under physiological conditions[110]. As protein import is dependent on the mitochondrial membrane potential, mitochondrial depolarization results in an accumulation of PINK1 on the outer membrane; the PINK1-mediated phosphorylation of certain proteins on the outer membrane mediates recruitment of the E3 ligase, PARKIN[111–114], to the outer membrane. PARKIN ubiquitylates lysine residues in the N-termini of mitochondrial outer membrane proteins, such as MFN1 and MFN2, thereby targeting the mitochondria for degradation by autophagosomes[115–119].

Several pathways regulate mitophagy (FIG. 5). Proteins that are important for autophagy, such as ULK1 and ULK2, can mediate mitophagy under different stimuli[120]. For example, when nutrients are sufficient, AMPK is inhibited and mTOR inhibits ULK1, suppressing mitophagy[121]. During nutrient deprivation, AMPK is activated and inhibits mTOR, facilitating ULK1 activation and mitophagy[120] (FIG. 3). Under oxidative stress, AMPK can be activated and inhibit mTOR, again stimulating mitophagy[55,121]. A more direct role for AMPK in the activation of mitophagy has also been suggested[122], whereby AMPK directly phosphorylates MFF on Ser155 and Ser172, triggering fission and, subsequently, mitophagy[123]. However, external stimuli that trigger this pathway are unknown and more research is needed.

Other stimuli, such as hypoxia, cause the Ser/Thr protein phosphatase phosphoglycerate mutase family member 5 (PGAM5) to dephosphorylate its substrate, the mitophagy receptor FUN14 domain-containing protein 1 (FUNDC1)[124]. FUNDC1 then interacts with microtubule-associated protein 1 light chain 3 (LC3), which mediates the formation of an autophagic membrane[124,125]. Alternatively, hypoxia can induce mitophagy through the actions of BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein X (NIX; also known as BNIP3L) via a mechanism involving HIF1α[126,127]. HIF1α can directly induce the transcription

of *BNIP3* and *NIX* by binding to the promoter of *BNIP3* and by recruiting other co-activator proteins to *NIX*. NIX and BNIP3 are transmembrane proteins located in the mitochondrial outer membrane and can activate mitophagy by dissipating the mitochondrial membrane potential and interacting with LC3 to deliver mitochondria to the autophagosome[127–130]. BNIP3 and NIX are also apoptotic regulators that can induce cell death or autophagy by increasing the production of ROS, by binding to pro-apoptotic proteins of the BCL-2 family, or by inhibiting the GTP-binding protein RHEB, an upstream activator of mTOR[131–133]. Previous studies suggest that crosstalk exists between both of the mechanisms that can regulate mitophagy[127,134,135], although the mechanisms of this proposed crosstalk are unclear and additional studies are needed to determine the mechanisms that regulate mitophagy in renal disease.

Mitochondria and renal diseases

Diseases such as AKI and diabetic nephropathy can cause an imbalance in mitochondrial homeostasis, negatively impacting mitochondrial energetics and the production of ATP. Much research supports a role for mitochondrial dysfunction in a number of renal diseases[136]. We focus on AKI and diabetic nephropathy as examples of how mitochondrial dysfunction can negatively affect mitochondrial energetics to contribute to disease progression.

Acute kidney injury

The outcome of AKI is renal dysfunction, as indicated by an increase in blood urea nitrogen (BUN) and serum creatinine level, and/or reduced urinary output[137]. Current treatment for AKI is lacking owing to its complex pathogenesis[138,139]. Over the past two decades, the incidence of AKI has increased; furthermore, the mortality rate for patients requiring renal replacement therapy is >60%[137,140–143]. Ultimately, unresolved AKI can cause long-term damage to the kidney, increasing the risk of chronic kidney disease (CKD)[144]. AKI can be categorized as prerenal, postrenal or intrinsic[139], and can result from sepsis, IRI, exposure to nephrotoxic reagents, trauma[145] or in response to decreased cardiovascular function[146,147]. One of the main sites of injury in AKI is the proximal tubules, where injury is characterized by disrupted brush borders and tight junctions, cell sloughing, apoptosis, necrosis and the subsequent backleak of filtrate across injured proximal tubular cells[148].

Much research has been conducted on mitochondrial dysfunction as an initiator of and contributor to AKI and as a therapeutic target[149]. Histologically, mitochondrial swelling and fragmentation are observed after diverse insults to the kidney[150]. A decrease in ATP production, an increase in ROS production, the release of cytochrome *c*, and the disruption of mitochondrial cristae are also observed, supporting a role for mitochondria in AKI[150]. A decrease in ATP production and mitochondrial dysfunction has been documented in many animal models of AKI, including sepsis, and these outcomes result from the loss of mitochondrial respiratory proteins in proximal tubules[151–153]. Furthermore, the loss of ETC proteins is persistent in the damaged kidney and might contribute to the slow recovery of renal function after AKI[151].

A number of factors in the ischaemic kidney disrupt the oxidation and transport of fatty acids, causing an accumulation of fatty acids in the cytoplasm and contributing to the decrease in ATP production and mitochondrial energetics[154,18,150,155,156]. For example, cofactors, such as NAD^+, are necessary for fatty acid oxidation, but a dysfunctional ETC is not able to regenerate NAD^+ (REF. 157). IRI also decreases the activity of CPT1 (REFS 18,158), the rate-limiting enzyme in the carnitine shuttle that transports fatty acids from the cytoplasm into the mitochondria[158], which decreases the transport of fatty acids into the mitochondria and reduces β-oxidation[158].

Increased levels of lactate and pyruvate and of hexokinase activity in the kidney have been reported after IRI, suggesting that an increase in glycolysis occurs after injury[159,160]. Increased levels of glycolytic enzymes have also been detected in injured renal tubules after IRI[161,162], suggesting that the kidney can respond to injury by altering its metabolic substrates to maintain function[163]. Further studies are needed to explore how this increase in glycolysis affects mitochondrial function in the kidney and if this change in metabolism contributes to long-term recovery following IRI.

Changes in mitochondrial dynamics also contribute to the decrease in mitochondrial energetics following AKI[164] (FIG. 6). The translocation of DRP1 into the mitochondrial outer membrane occurs shortly after kidney injury[151,165], and activation of DRP1 in ischaemic kidneys promotes mitochondrial fragmentation and apoptosis[166]. Loss of cristae structure is also observed in AKI, which dissipates the mitochondrial membrane potential and halts ATP production[150]. Administration of a pharmacological inhibitor of DRP1, mdivi-1, protected kidneys from AKI by inhibiting mitochondrial fragmentation, supporting a role for altered mitochondrial dynamics in AKI[165].

Mitophagy is also activated after ischaemic AKI. In mice from which the genes encoding the autophagy regulators autophagy-related protein 7 (ATG7) and ATG5 were specifically knocked out in renal proximal tubules, mitochondrial dysfunction was greater in renal proximal tubules in response to IRI, as characterized by severe morphological changes, increased ROS production and apoptosis[167–169]. Activation of NIX and BNIP3 causes the release of ROS and the pro-apoptotic proteins BAX and BAK, in hypoxic conditions[116,170]. Deletion of *BAX* and *BAK* in mouse kidneys not only protected mice from ischaemic AKI but also suppressed mitochondrial fragmentation and the release of cytochrome *c*, preserving mitochondrial integrity[171]. A lack of ATG7 also exacerbated cisplatin-induced AKI in mice[134,167]. These studies suggest that crosstalk occurs between components of the cell death machinery and the autophagy machinery in the activation of mitophagy.

In mouse models of AKI, the transcription and protein expression of PGC1α are persistently suppressed, but are eventually restored to basal levels with

Mitochondrial cristae
Folds in the mitochondrial inner membrane that increase the surface area for mitochondrial respiration to take place.

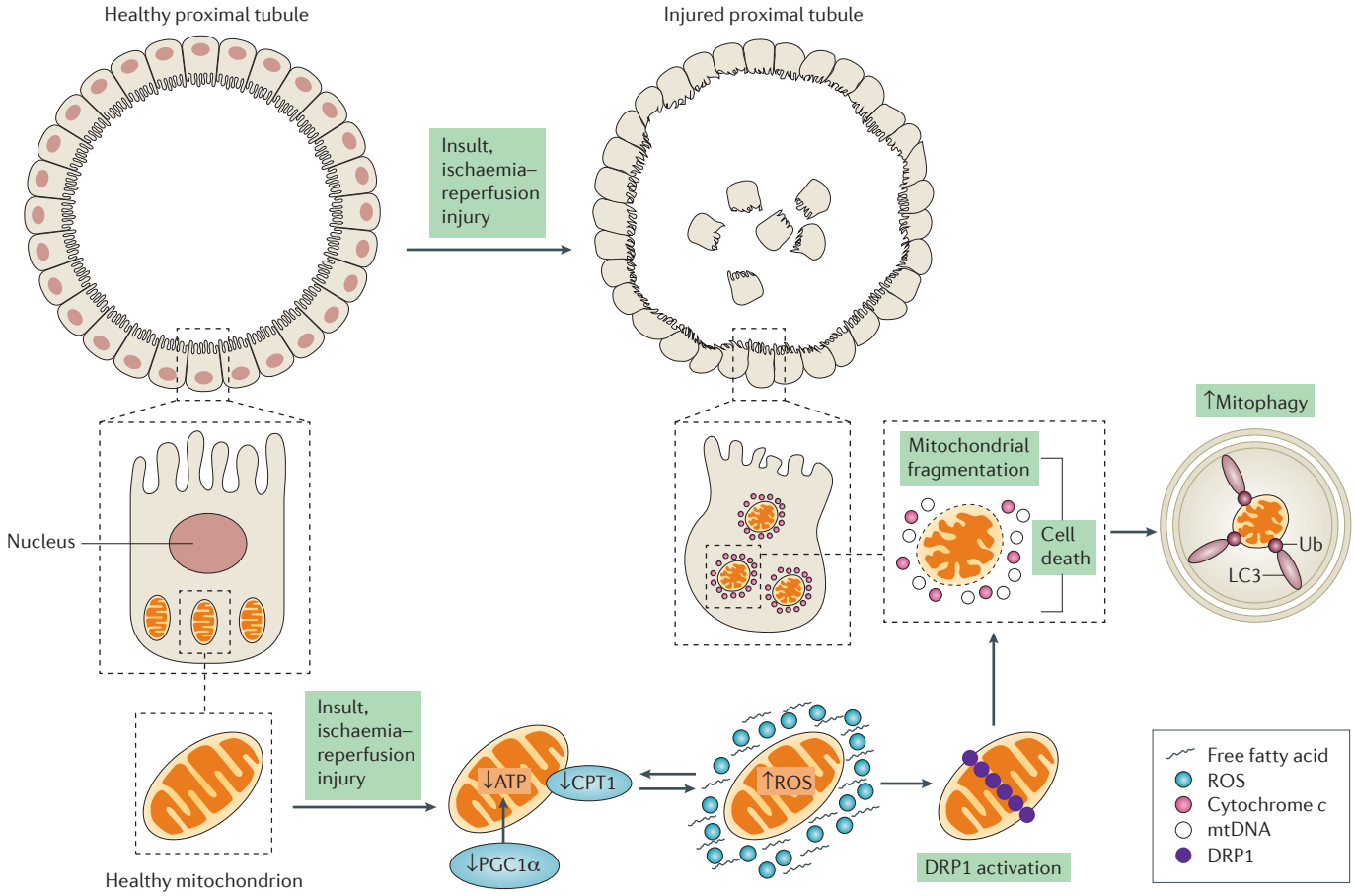

Figure 6 | **Changes in mitochondrial morphology lead to tubular damage in acute kidney injury.** A healthy proximal tubule consists of an intact brush border with tight junctions and contains a network of mitochondria to maintain its function. After ischaemia–reperfusion injury (IRI), changes in mitochondrial function and morphology lead to mitochondrial dysfunction, and eventually to injured proximal tubules. In the early stages of acute kidney injury (AKI), a number of events may happen concurrently to cause a decrease in ATP production. These events include a decrease in the expression of carnitine *O*-palmitoyltransferase 1 (CPT1) (causing fatty acid accumulation and decreasing β-oxidation for ATP production), a decrease in the expression of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) and an increase in the production of reactive oxygen species (ROS) (bidirectional arrows). Together, these events can trigger the activation and accumulation of dynamin 1-like protein (DRP1) on the mitochondrial outer membrane, promoting mitochondrial fragmentation and eventually cell death. The release of cytochrome *c* and mitochondrial DNA (mtDNA) from dysfunctional mitochondria causes an increase in mitophagy. Mitochondrial dysfunction can induce cell death in injured proximal tubules, resulting in the loss of nuclei and tight junctions and in disrupted brush borders. Apoptotic or necrotic tubules can lead to cell sloughing, as seen in the centre of the tubule.

recovery[151]. As PGC1α can regulate the transcription of mitochondrial proteins, the level of these proteins is also decreased after AKI[151,172]. In a model of septic AKI, global *PPARGC1A*-knockout mice showed a greater increase in BUN and creatinine levels than did wild-type mice[152]. Renal-specific *PPARGC1A*-knockout mice exhibited persistent AKI in response to sepsis[152]. By contrast, overexpression of PGC1α in renal proximal tubule cells attenuated oxidant injury *in vitro*[59]. Together, these studies show that PGC1α is necessary for the recovery of renal function in AKI.

Investigations into the mechanisms by which PGC1α regulates the recovery from AKI revealed a role for PGC1α in NAD biosynthesis. The levels of nicotinamide, a precursor for NAD, were decreased after AKI in *PPARGC1A*-knockout mice, and supplementation with nicotinamide reversed ischaemic AKI[173]. We have reported that drugs or chemicals can upregulate mitochondrial biogenesis by increasing the expression of PGC1α in the recovery phase following IRI through two G-protein coupled receptors (GPCRs): the β_2 adrenergic receptor and the 5-hydroxytryptamine 1F receptor[174,175] (see below).

The role of SIRT3 in cisplatin-induced AKI has also been explored. SIRT3 is a mitochondrial-specific protein deacetylase with an active role in mitochondrial function and integrity[176]. An *in vitro* study using cisplatin-injured human renal proximal tubules showed that the overexpression of SIRT3 decreased the translocation of DRP1 from the cytosol to the mitochondrial outer membrane

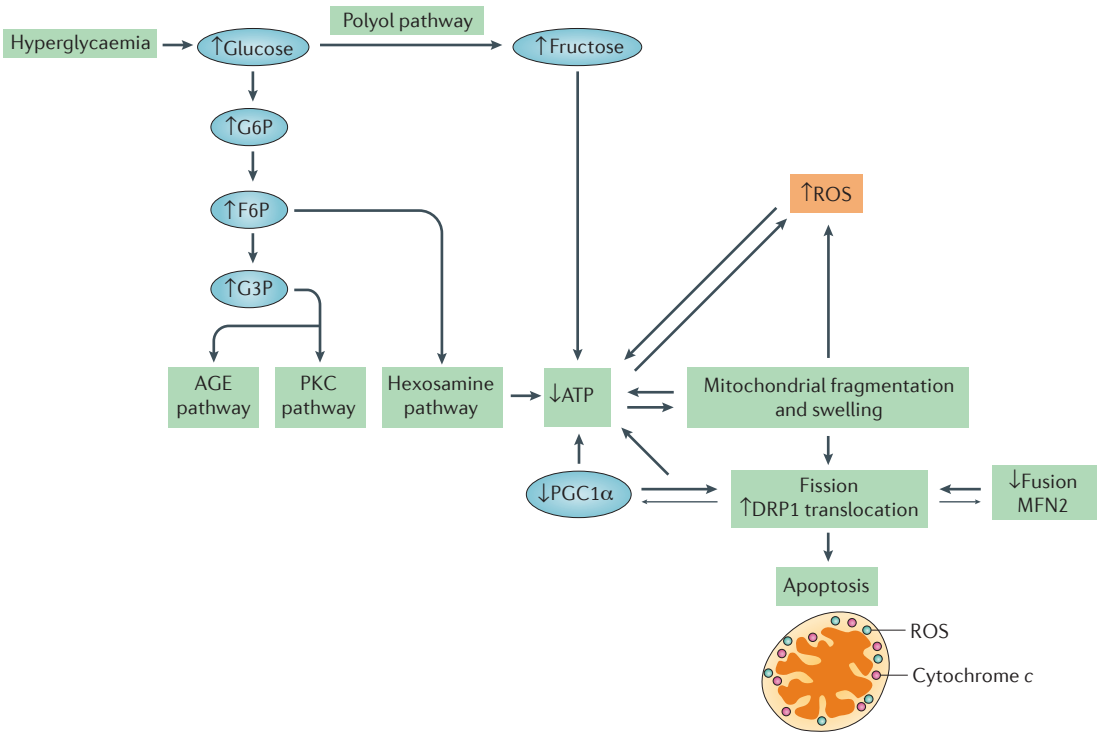

Figure 7 | **Factors contributing to mitochondrial dysfunction in diabetic nephropathy.** Hyperglycaemia is the primary contributing factor to mitochondrial dysfunction in diabetic nephropathy. An increase in glucose level results in an increase in glycolysis, in turn activating the advanced glycation end product (AGE) pathway, the protein kinase C (PKC) pathway and the hexosamine pathway, which results in a decrease in ATP levels. Hyperglycaemia also activates the polyol pathway, which increases fructose levels and, consequently, decreases ATP levels. Mitochondrial fragmentation and swelling is observed in early diabetic nephropathy, leading to an increase in fission and the production of reactive oxygen species (ROS). The correlations between increased mitochondrial fragmentation and decreased ATP, and between ROS production and decreased ATP, are interdependent. Whether one causes the other is unclear, as depicted by the bidirectional arrows. Decreases in the levels of mitofusin 2 (MFN2) and peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) correlate with, and might contribute to, the increase in mitochondrial fission observed in diabetic nephropathy, as indicated by the larger arrows pointing towards increased mitochondrial fission. Decreases in mitochondrial energetics that are caused by changes in mitochondrial morphology and hyperglycaemia lead to apoptosis in diabetic nephropathy. F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G3P, glyceraldehyde-3-phosphate.

and thus decreased mitochondrial fission, supporting a role for SIRT3 in regulating mitochondrial dynamics after AKI[176]. Deletion of *SIRT3* exacerbates injury in a cisplatin-induced AKI mouse model, supporting its role in recovery from AKI[176].

Diabetic nephropathy

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in the USA[177,178]. It is characterized by hyperglycaemia, albuminuria, the accumulation of extracellular matrix proteins, and glomerular and tubular epithelial hypertrophy, as well as a reduced glomerular filtration rate following an initial period of hyperfiltration[179]. Mitochondrial energetics are altered in diabetic nephropathy owing to increased ROS and hyperglycaemia[180], both of which induce changes in the ETC that cause a decrease in ATP production and an increase in apoptosis[180]. In line with these observations, increased fission, mitochondrial fragmentation and reduced levels of PGC1α are all observed in the early stages of diabetes mellitus[181,182]. Structural changes in mitochondria correlate with the observed changes in mitochondrial energetics[182].

Hyperglycaemia is the main factor that contributes to the development of diabetic nephropathy (FIG. 7). Hyperglycaemia increases the production of NADH and $FADH_2$ by the TCA cycle, fueling the ETC[183]. ROS released from the ETC can damage mtDNA, hindering the production of mitochondrial proteins[183]. The hyperglycaemic state was originally thought to cause mitochondrial dysfunction by stimulating the development of hyperpolarized mitochondria, which produce more ATP and release higher levels of superoxide from complexes I and III than healthy mitochondria[180,184,185]. Administration of antioxidants such as vitamin E and vitamin A did not, however, attenuate the complications of patients with diabetes mellitus, suggesting that mitochondrial ROS might not be the primary mediator of mitochondrial dysfunction in diabetic nephropathy[186]. Hyperglycaemia can also increase the level of advanced glycation end products (AGEs), and the activity of the protein kinase C (PKC) and hexosamine pathways, which can contribute to mitochondrial dysfunction[187]. All three events cause deleterious effects that include increased fibrosis, thrombosis, oxidative damage and abnormalities in the vasculature and in blood flow[187].

Hyperglycaemia also stimulates the conversion of glucose to fructose via the polyol pathway in proximal tubules, leading to ATP depletion[188]. A role for endogenous fructose metabolism in the regulation of diabetic nephropathy was suggested by a study showing that deleting the gene that encodes ketohexokinase (KHK; also known as hepatic fructokinase) — the enzyme responsible for the conversion of fructose to fructose-1-phosphate — protected mice from streptozotocin-induced diabetic nephropathy[189]. Proximal tubules are a major site of ketohexokinase expression[188,190] and ATP levels were increased and tubular morphology was improved in diabetic *Khk*$^{-/-}$ mice compared with that of diabetic wild-type mice, suggesting a role for fructose metabolism in the pathogenesis of diabetic nephropathy[189].

Mitochondrial fragmentation has been observed in proximal tubules in the early stages of diabetes mellitus[181], although the mechanisms that drive changes in mitochondrial dynamics in diabetes are not yet clear. Fission dissipates the mitochondrial membrane potential, decreasing the production of ATP and promoting apoptosis[191]. Several studies have suggested a role for RHO-associated protein kinase 1 (ROCK1) signalling in activating fission in the diabetic kidney[192]. ROCK1 promotes the translocation of DRP1 to the mitochondria and triggers fission by phosphorylating DRP1 (REF. 192). Deletion of *ROCK1* in mice with streptozotocin-induced diabetes prevents mitochondrial fission, attenuates the increase in ROS production and restores bioenergetic function in the kidney[192].

Patients with diabetes mellitus have reduced levels of the fusion protein MFN2[193]. In line with this finding, kidney-specific overexpression of MFN2 protects rats from streptozotocin-induced diabetic nephropathy[193]. MFN2 overexpression decreased ROS production, decreased kidney volume and attenuated the pathological changes seen in the diabetic kidney[193]. Induced in high glucose 1 (IHG1; also known as THG1L) is another protein that is involved in mitochondrial fusion and has been shown to regulate mitochondrial dynamics and biogenesis in the diabetic kidney[194]. IHG1 can enhance the ability of MFN2 to bind to GTP and interacts directly with MFN2 to mediate fusion[194]. Inhibition of IHG1 reduces ATP production and hinders fusion *in vitro*[194]. IHG1 also stabilizes PGC1α activation[195].

Reduced levels of PGC1α have also been observed in diabetic rat kidneys[196]. The overexpression of PGC1α in mesangial cells *in vitro* attenuated the pathophysiological changes induced by hyperglycaemic conditions[196]. The decrease in mitochondrial biogenesis in diabetic rat kidneys is consistent with the translocation of DRP1 to the mitochondrial outer membrane and an increase in mitochondrial fragmentation[196]. The levels of PGC1α mRNA and protein were also reduced in podocytes that were cultured under hyperglycaemic conditions compared with the levels in podocytes that were cultured under normal glucose conditions, indicating a decrease in mitochondrial biogenesis[197].

Another study has described an important role for pyruvate kinase M2 (PKM2) in diabetic nephropathy. The expression and activity of PKM2 is upregulated in patients with long-term diabetes mellitus who have not developed diabetic nephropathy but not in patients with diabetic nephropathy[198]. Podocytes from *PKM2*-knockdown mice have decreased *PPARGC1A* mRNA and mitochondrial mass, whereas activation of PKM2 attenuated the decrease in mitochondrial function and glycolytic flux in podocytes *in vitro*. *In vivo* studies showed that activation of PKM2 in mice attenuated the diabetes-induced decrease in *PPARGC1A* mRNA and increased the expression of OPA1, increasing mitochondrial fusion[198]. Activation of PKM2 can therefore reverse mitochondrial dysfunction and renal abnormalities associated with diabetes mellitus. These studies highlight the need for further research in this area, as targeting the balance between mitochondrial biogenesis and dynamics could be a potential therapeutic approach for diabetic nephropathy.

Mitochondrial energetics and therapy

Targeting AMPK signalling

AMPK signalling has been implicated as a target for correcting metabolism and mitochondrial function, especially in the kidney. As mentioned above, AMPK is a metabolic sensor of ATP in the cell. A high AMP:ATP ratio activates AMPK to stimulate cell growth and cellular metabolism. The AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-riboside (AICAR), prevents glomerulopathy and tubulointerstitial fibrosis in mice by stimulating fatty acid oxidation[199] (TABLE 1). AICAR also has a therapeutic effect in mouse renal IRI and can improve glucose utilization in obese, insulin-resistant rats[200,201]. The activation of AMPK by AICAR increased the level of PGC1α and mitochondrial proteins while reducing ROS production in a diabetic mouse model[202].

Several studies have suggested that crosstalk exists between AMPK and SIRT3 signalling[203,204]. SIRT1 and SIRT3 are activated by NAD^+ (REF. 205). Cisplatin-treated mice have decreased expression of *Sirt3* and lower SIRT3 protein levels, increased tubular damage, and decreased levels of phosphorylated AMPK compared with that of saline-treated control mice[206]. Administration of AICAR to cisplatin-treated mice attenuated the decrease in SIRT3 expression, phosphorylated AMPK level, and tubular damage[206]. These studies provide a therapeutic rationale for targeting AMPK signalling in the kidney to improve outcomes in AKI and diabetic nephropathy.

Targeting PPARs

PPARs can regulate cellular metabolism, mitochondrial function, mitochondrial biogenesis, fatty acid oxidation and glucose homeostasis; thus, targeting them could be beneficial for patients with renal disease related to mitochondrial dysfunction.

Activation of PPARs can ameliorate ischaemic AKI[207–209]. As discussed above, an accumulation of fatty acids and increased ROS production can decrease the efficiency of the ETC. Defects in fatty acid oxidation have been attributed to the downregulation of PPARs during renal ischaemia[18]. Fenofibrate, which is used to treat dyslipidaemia, activates PPARα[210] (TABLE 1). Activation of PPARα leads to activation of lipoprotein lipase, which

Streptozotocin
A glucosamine-nitrosourea that is used to induce experimental diabetes in animals by specifically targeting and damaging beta cells.

Dyslipidaemia
Abnormalities in lipoprotein metabolism, resulting in elevated or deficient levels of lipids and/or lipoproteins in the body.

Table 1 | **Approaches to correct abnormal mitochondrial function in AKI and diabetic nephropathy**

Agent	Mechanism of action	*In vivo* and clinical studies*
Acute kidney injury		
AICAR (an AMPK activator)	• Increases AMPK activation • Increases crosstalk between SIRT3 and AMPK	• AICAR administration attenuated decreased serum creatinine and urea levels in Sprague–Dawley rats with IRI (2012)[200] • AICAR attenuated BUN and serum creatinine levels in cisplatin-treated mice (2015)[206]
Formoterol (a β_2AR agonist)	Binds to β_2AR and induces mitochondrial biogenesis	Formoterol restored mitochondrial and renal function in mice with IRI within 6 days (2014)[174]
LY344864 (a 5-HT_{1F} receptor agonist)	Binds to 5-HT_{1F} and induces mitochondrial biogenesis	LY344864 restores renal function in mice with IRI within 6 days (2014)[175]
Elamipretide (a Szeto–Schiller peptide (specifically SS-31))	Prevents the peroxidation of cardiolipin by cytochrome *c*	• Enhances efficiency of the ETC and prevents mitochondrial swelling in rats (2014)[218] • Phase I study (NCT02436447) in patients with impaired renal function (2015)[221]
Diabetic nephropathy		
AICAR (an AMPK activator)	Increases glucose utilization	AICAR decreased blood glucose levels in *db/db* diabetic mice and *ob/ob* obese mice (2002)[228]
Fenofibrate (a PPARα agonist)	• Decreases hyperglycaemia • Increases free fatty acids by targeting lipase • Decreases dyslipidaemia and albuminuria	• Corrected glucose homeostasis in *db/db* diabetic mice (2006)[213] • Decreased serum creatinine levels and had a renoprotective role for diabetic nephropathy in diabetic rats (2016)[215] • Administrating fenofibrate to patients with type 2 diabetes mellitus decreased cardiovascular disease events (2005)[229]

5-HT_{1F}, 5-hydroxytryptamine receptor 1F; β_2AR, β_2 adrenergic receptor; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-riboside; AKI, acute kidney injury; AMPK, AMP-activated protein kinase; BUN, blood urea nitrogen; ETC, electron transport chain; IRI, ischaemia–reperfusion injury; PPARα, peroxisome proliferator-activated receptor-α; SIRT3, sirtuin 3. *The year of the clinical study is given in parentheses.

hydrolyses triglycerides into glycerol and free fatty acids for metabolism[210]. PPARs can also stimulate mitochondrial biogenesis; for example, compounds such as bardoxolone increase the level of *PPARG* (encoding PPARγ) and *NFE2L2* (encoding NRF2) mRNA, leading to mitochondrial biogenesis[211]. However, the use of bardoxolone in clinical trials for patients with type 2 diabetes mellitus and stage 4 CKD showed adverse effects in patients, including an increase in the rate of heart failure events, resulting in termination of the trial[212].

The efficacy of PPAR agonists in animal models suggests these agents could show promise for the treatment of diabetic nephropathy. Treatment of *db/db* diabetic mice with fenofibrate led to decreased hyperglycaemia and insulin resistance, potentially by correcting glucose homeostasis[213]. Studies have also shown that treatment of diabetic mice with fenofibrate leads to a decrease in fatty acids in the kidney, supporting its potential as a therapeutic for diabetic nephropathy[214–216]. These *in vivo* studies provide evidence that fenofibrate might be suitable for the treatment of patients with diabetic nephropathy. Indeed, fenofibrate decreased dyslipidaemia and albuminuria in patients with type 2 diabetes mellitus and reduced the risk of further cardiovascular events[217]. Taken together, these studies confirm that PPARs have a role in diabetic nephropathy and are a therapeutic target.

Targeting G protein-coupled receptors

Although a wide variety of GPCRs are expressed in the kidney, few studies correlate GPCRs with mitochondrial function in the kidney and other organs. We proposed that compounds that target two different GPCRs — β_2 adrenergic receptor (β_2AR) and 5-hydroxytryptamine receptor 1F (5-HT_{1F}) — can induce mitochondrial biogenesis, restore mitochondrial function and stimulate the recovery of renal function following IRI. Formoterol, a β_2AR agonist used to treat asthma and chronic obstructive pulmonary disease, stimulates mitochondrial biogenesis and the expression of PGC1α in renal proximal tubular cells in mice[174]. The administration of formoterol in a model of IRI accelerated the recovery of mitochondrial and renal function by 6 days[174]. LY344864 is a potent 5-HT_{1F} agonist; it induced mitochondrial biogenesis in naive mice and accelerated the recovery of mitochondrial biogenesis and renal function in the same AKI model[175]. Several GPCR ligands, such as atrasentan, are currently in clinical trials of diabetic nephropathy; however, whether they act by influencing mitochondrial energetics is unknown and requires further research. These studies provide a foundation for pursuing the targeting of GPCRs, particularly β_2AR and 5-HT_{1F}, as a treatment for mitochondrial dysfunction in renal diseases.

Using mitochondrial peptides

A 2014 study described a family of peptides, called Szeto–Schiller peptides (SS peptides), which specifically target cytochrome *c* activity in the ETC, enhancing its efficiency and increasing 'state 3 respiration' — that is, ATP production in the presence of excess substrates and ADP[218]. SS peptides are highly polar, water-soluble tetrapeptides that can cross the blood–brain barrier and specifically target the inner mitochondrial membrane. The SS peptides do not cause mitochondrial depolarization upon entry, making these compounds highly promising for treatment. SS peptides prevent the peroxidation of cardiolipin,

a phospholipid that is important for maintaining cristae formation, by cytochrome *c*[218]. Cytochrome *c* binds to and oxidizes cardiolipin, disrupting cristae formation and detaching cytochrome *c* from the inner mitochondrial membrane[219,220]. The SS-31 peptide (also known as elamipretide) has been shown, in a variety of animal disease models, especially in AKI, to promote ATP recovery and cristae formation[218]. Pretreatment of rats with SS-31 *in vivo* maintained cristae formation and prevented mitochondrial swelling of renal tubular epithelial cells[218]. Due to the success in animal models, SS-31 is currently in clinical trials for the treatment of impaired renal function[221] (TABLE 1).

Conclusions

Mitochondrial homeostasis involves a network of cellular processes that regulate ATP production; the disruption of these processes can result in mitochondrial dysfunction and organ damage. Although much is known about mitophagy and mitochondrial fission, fusion and biogenesis, the precise role of these processes in renal disease remains to be determined. It is clear, however, that mitochondrial dysfunction is common and occurs early in AKI and diabetic nephropathy. Furthermore, the absence of recovery of mitochondrial function after diverse insults might lead to the continued impairment of renal function, leading to CKD. As renal cell repair and the recovery of renal function is dependent on the ability of mitochondria to produce ATP, restoring mitochondrial function might reverse cellular injury and restore renal function, particularly for diseases such as AKI and diabetic nephropathy. Collectively, the available studies corroborate the need to target mitochondrial homeostasis to restore mitochondrial function and stimulate organ repair or prevent further declines in organ function.

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Both authors researched data for the article, made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.

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