Mitochondria as a therapeutic target for common pathologies

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Abstract | Although the development of mitochondrial therapies has largely focused on diseases caused by mutations in mitochondrial DNA or in nuclear genes encoding mitochondrial proteins, it has been found that mitochondrial dysfunction also contributes to the pathology of many common disorders, including neurodegeneration, metabolic disease, heart failure, ischaemia–reperfusion injury and protozoal infections. Mitochondria therefore represent an important drug target for these highly prevalent diseases. Several strategies aimed at therapeutically restoring mitochondrial function are emerging, and a small number of agents have entered clinical trials. This Review discusses the opportunities and challenges faced for the further development of mitochondrial pharmacology for common pathologies.

Mitochondrial permeability transition pore
(MPTP). The MPTP is a large conductance pore that opens in the mitochondrial inner membrane in response to oxidative stress and elevated calcium levels. This leads to mitochondrial swelling and cell death.

Reactive oxygen species
(ROS). ROS such as superoxide and hydrogen peroxide are produced as a by-product of normal metabolism. They can cause nonspecific oxidative damage to proteins, DNA and lipids that contributes to pathologies and can also act as redox signals.

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Mitochondria perform many key roles in the cell, most notably oxidative phosphorylation, central carbon metabolism and the biosynthesis of intermediates for cell growth, but they are also responsible for several other essential processes that determine cell function and fate[1–7] (BOX 1; FIG. 1). Consequently, mutations in nuclear or mitochondrial DNA (mtDNA) genes that disrupt mitochondrial function lead to devastating 'primary' mitochondrial diseases[1,3,8–11]. Our knowledge of how mitochondria function in the cell has expanded dramatically. It is now clear that mitochondria participate in nearly all aspects of cell function, affecting processes not traditionally linked with the organelle, including cancer, inflammation, metabolic signalling and cell death, transformation and fate[5–7]. Hence, mitochondrial dysfunction has been found to contribute to many common disorders, including neurodegeneration, metabolic disease and heart failure (HF)[4,5,12,13]. These 'secondary' mitochondrial diseases can arise even if the proximal cause is not mitochondrial (for example, when the initiating disease process disrupts mitochondrial function as a downstream effect)[6,7,10,12,14–16]. Thus, drugs designed to act on mitochondria may be effective therapies for a range of common diseases and could be more effective than when applied to the notoriously hard-to-treat diseases that arise owing to mutations in mitochondrial genes[3,7,10,12,14]. Importantly, drugs designed to affect mitochondrial function can be applied to many highly prevalent diseases and pathological processes, with important social, medical and economic impacts[2,17,18]. In many cases, progress in developing new therapeutic approaches for these common diseases has been dispiritingly slow, as is illustrated by the lack of new drugs coming to market for stroke or neurodegenerative diseases. Focusing on mitochondria offers a promising alternative approach to developing new therapeutic options for these disorders[14,19,20].

Examples of mitochondrial agents that are currently being, or have recently been, assessed in humans include agents to replenish NAD^+ pools such as nicotinamide mononucleotide (NMN)[21], mitochondria-targeted protective compounds such as MitoQ[22,23] and Bendavia (SS31)[24], antioxidants such as coenzyme Q_{10} (CoQ_{10})[25] and cyclosporin A (CsA), an inhibitor of the mitochondrial permeability transition pore (MPTP)[26,27]. Given that the development and application of drugs designed to affect mitochondria are still in their infancy, this Review focuses on the general principles, vast potential and ongoing challenges for intervening at the mitochondrial level.

Rationale for targeting mitochondria

Disruption to mitochondrial bioenergetic and metabolic function can lead to many secondary mitochondrial disorders (FIG. 1). Interestingly, common patterns regarding how mitochondria contribute to the aetiology of disparate pathologies have emerged[5,14,28]. Important among these are the aberrant production of reactive oxygen species (ROS), calcium dyshomeostasis, defective mitochondrial biogenesis, disruption to mitochondrial dynamics and quality control, necrotic cell death through induction of the MPTP, inappropriate activation or suppression of apoptosis, lowered cellular ATP:ADP ratio, decreased NAD^+ levels and alterations in mitochondrial signalling pathways[7,14,28,29] (FIG. 1). In many cases, these different types of organelle dysfunction are linked mechanistically, hence are often found together, and they may contribute to disease by acute,

irreversible cell death, long-term disruption to the role of mitochondria as signalling hubs or the lifelong accumulation of environmental damage that leads to a degenerative disorder[15]. The details of how mitochondrial dysfunction leads to specific pathologies are discussed below.

In short, there are three factors supporting the pursuit of mitochondria as a therapeutic target for common pathologies. First, many prevalent diseases are secondary mitochondrial disorders in that mitochondrial dysfunction contributes to the disease process or clinical progression. Hence, targeting the organelle can improve patient outcome, although mitochondrial dysfunction may not be the primary driver of pathology. Second, mitochondria contribute to diverse pathologies through common pathways[10,14]; therefore, a single therapeutic approach may apply to multiple disorders. Finally, the common diseases in which targeting mitochondria shows promise are of increasing medical, social and economic impact in our ageing population. Given that the development of new drugs for these disorders has been frustratingly slow, new approaches are needed[30–32].

Box 1 | **Mitochondrial biogenesis, oxidative phosphorylation and metabolism**

Mitochondria are assembled through the interplay between the nuclear and mitochondrial genomes. Mammalian mitochondrial DNA (mtDNA) encodes 37 genes — 13 for polypeptide components of the oxidative phosphorylation machinery as well as the 22 tRNAs and 2 ribosomal RNAs (rRNAs) required for their transcription and translation within the organelle[1–6]. Mitochondria contain ~1,500 types of protein that are encoded on the nuclear genome, translated on cytoplasmic ribosomes and then imported into mitochondria by the translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes[14,15]. Phospholipids are either synthesized in the organelle or imported after synthesis in the endoplasmic reticulum membrane[5,15]. The mitochondrial outer membrane is similar in composition to those in the rest of the cell and contains a pore formed by the β-barrel protein voltage-dependent anion channel (VDAC) that enables interchange between the intermembrane space and the cytosol[14]. The inner membrane contains a large amount of the phospholipid cardiolipin, and its area is greatly enhanced by infolding into cristae that are in the shape of flattened disc-like sacs with narrow necks that connect them to the intermembrane space[81]. The flattened shape is maintained by a line of F_oF_1-ATP synthase dimers whereas the neck structure and contact sites between the inner and outer membranes are maintained by the mitochondrial contact site and cristae organizing system (MICOS)[15]. The extensive surface area of the cristae is required for effective oxidative phosphorylation[2,5]. The rest of the inner membrane is called the boundary membrane and is the site of mitochondrial protein import[15].

Mitochondria are not isolated organelles but are a dynamic network within the cell, continually fusing and dividing[15,81,82]. Mitochondrial fusion is determined by proteins such as mitofusins (MFN1 and MFN2) and optic atrophy protein 1 (OPA1), whereas fission is controlled by proteins such as dynamin-related protein 1 (DRP1)[81,82]. Mitochondrial morphology is a balance between fusion and fission events, the latter being associated with contact sites to the endoplasmic reticulum, and are intimately linked to mitochondria quality control and the degradation of damaged mitochondria through mitophagy[15,81,82]. In addition, there are a number of proteases, lipases and nucleases that act within the organelle to degrade or repair internally damaged parts of the organelle[57,58,93]. Mitochondria can also package and bud off damaged material as mitochondria-derived vesicles[90–92].

The mitochondrial content of the cell is set by the balance of mitochondrial biogenesis and degradation, which requires the regulation of the expression of the nuclear and mitochondrial genomes in response to the metabolic and energy demands of the cell[81,82]. These processes are regulated by a range of transcription factors, such as nuclear respiratory factors (NRF1 and NRF2) in association with transcriptional co-activators such as peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α)[73,75,76,78]. The activity of these factors themselves are frequently modified by post-translational modification — for example, by the energy sensor AMP-activated kinase (AMPK), which, upon activation by a lowered ATP:ADP or ATP:AMP ratio, inhibits anabolic pathways and stimulates catabolic pathways[13]. Together, these regulatory pathways enable mitochondrial function to adapt to both the long-term and short-term requirements of the cell[2,5,14].

Energy metabolism is the core function of mitochondria. At its heart is the citric acid cycle (CAC), which takes the acetyl-CoA generated from the pyruvate provided by glycolysis and breaks the acetyl moiety down to carbon dioxide, with the electrons going to NADH in the matrix or to the coenzyme Q (CoQ) pool within the mitochondrial inner membrane, which comprises an oxide form ubiquinone (Q) and a reduced form ubiquinol (QH_2)[1–6,14] (FIG. 1). Fatty acids are also broken down by β-oxidation to acetyl-CoA with the electrons passed on to NADH or the CoQ pool. NADH transfers its electrons through complex I to the CoQ pool, which also receives electrons from many other sources[14]. From the CoQ pool, the electrons pass through complex III to cytochrome *c* before reducing oxygen to water at complex IV. The reduction potential difference driving electron movement through complexes I, III and IV is used to pump protons across the mitochondrial inner membrane, which builds up a protonmotive force (Δp) across the mitochondrial inner membrane comprising a membrane potential ($\Delta\psi$) of ~150–160 mV and a pH gradient of ~0.5 pH units, which is then used to drive ATP synthesis at the F_oF_1-ATP synthase[14,282]. The ATP is exported from the matrix to the cytosol in exchange for ADP by the adenine nucleotide exchanger (ANT), whereas the phosphate (P_i) is symported with H^+; therefore, mitochondrial ATP synthesis can drive ATP-dependent work in the cytosol[108].

In addition to energy metabolism, mitochondria are also central to many other metabolic pathways, synthesizing iron sulfur (FeS) centres, haem and CoQ, whereas the CAC is intimately involved in cellular amino acid and carbohydrate metabolism[15,72]. These core metabolic roles require the continual and selective transport of polar metabolites between the mitochondria and the cytoplasm, without proton permeation of the inner membrane, which would uncouple ATP synthesis[108]. Metabolite transport occurs through families of solute carriers in the inner membrane (for example, the SLC25 family[108]), whereas VDAC enables transport of a range of metabolites across the mitochondrial outer membrane[108].

Therapeutic approaches to mitochondria

There are a number of approaches aimed at modulating mitochondrial function in primary and secondary mitochondrial diseases[3,9]. These include behavioural interventions, such as changes in diet or exercise[33], exposure to hypoxia[34], stem cell therapies[35], replacing defective mtDNA in an oocyte[36] and supplementation of a tissue with exogenous mitochondria[37]. Furthermore, there are many potential therapeutic strategies utilizing gene therapies to deliver corrected versions of a defective gene or to ectopically express proteins designed to degrade mutated mtDNA[38] or alter metabolism[39]. Although all these approaches could lead to potential treatments for common pathologies, their coverage is beyond the scope

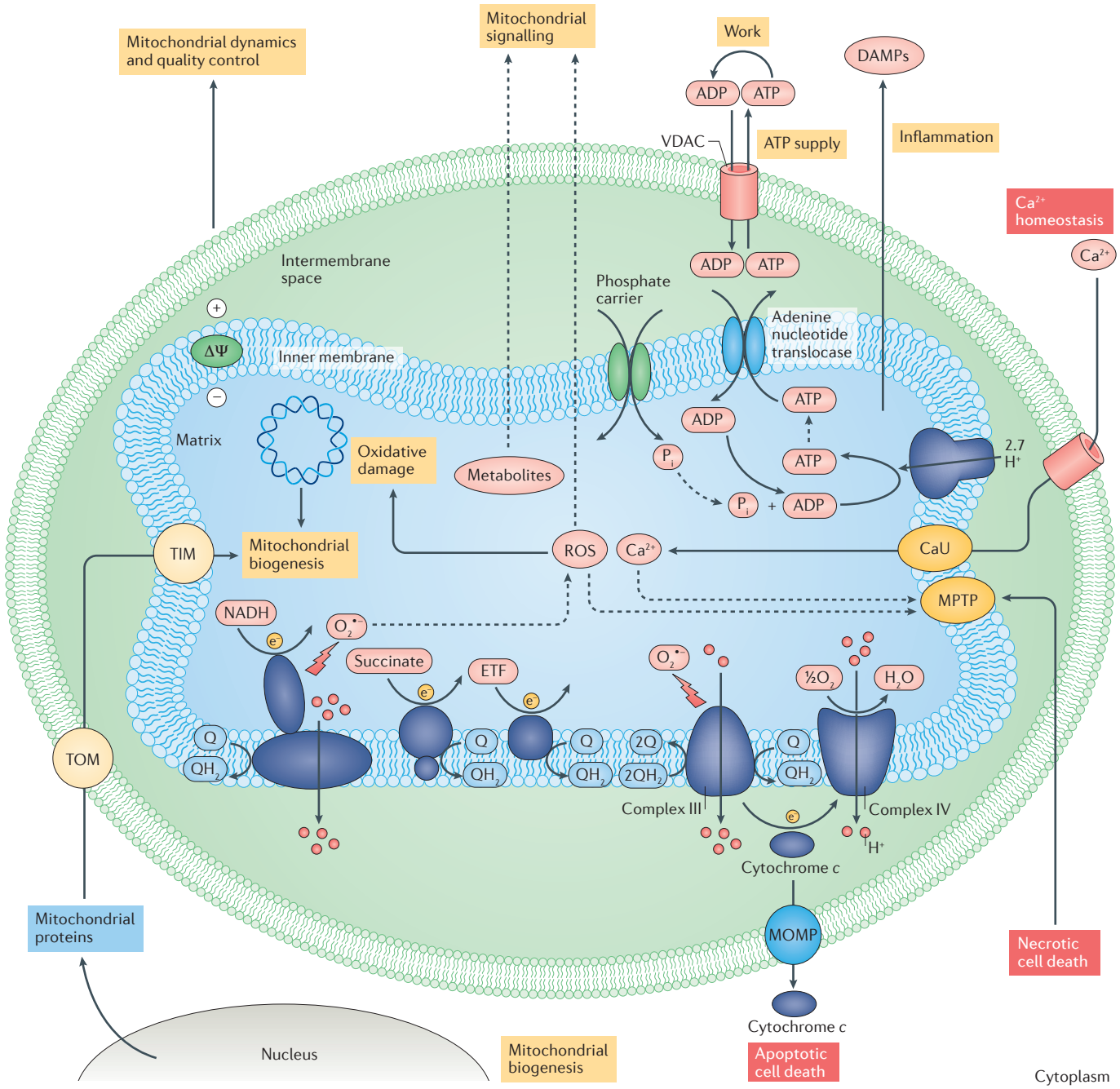

Figure 1 | **Mitochondrial function and pathological disruption.** The key roles of mitochondria are illustrated here and discussed further in BOX 1. Disruption to mitochondrial function can lead to pathology by affecting several pathways: ATP supply; mitochondrial biogenesis; mitochondrial fission and/or fusion and organelle quality control; reactive oxygen species (ROS) production; induction of the mitochondrial permeability transition pore (MPTP); release of pro-apoptotic factors to the cytosol by induction of mitochondrial outer membrane permeabilization (MOMP); activation of the innate immune system by release of damage-associated molecular patterns (DAMPs); mitochondrial signalling; and calcium homeostasis. Δp, protonmotive force; Δψ, membrane potential; CaU; calcium uniporter; e^-, electron; ETF, electron transfer flavoprotein; P_i, phosphate; Q, ubiquinone; QH_2, ubiquinol; TIM, translocase of the inner membrane; TOM, translocase of the outer membrane; VDAC, voltage-dependent anion channel.

of this Review, which focuses on the general strategies for the development of small-molecu le therapies that can modulate mitochondrial function.

Drugs can act directly on the mitochondria themselves or affect the organelle indirectly by binding to regulatory targets in the cytosol or nucleus[14,40]. An important aspect of drugs that affect the organelle directly is the ability to selectively target bioactive moieties to mitochondria in vivo by conjugation to lipophilic cations or to peptides, which facilitates drug effectiveness by enhancing potency, avoiding side effects and accelerating delivery[14,20,41,42] (BOX 2).

There are five broad therapeutic strategies in which small molecules can be used to affect mitochondria directly or indirectly in secondary mitochondrial diseases: repairing or preventing damage to the organelle; inducing mitochondrial biogenesis; enhancing organelle quality control by stimulating degradation of damaged mitochondria or organelle components; co-opting mitochondrial function to induce cell death; or altering mitochondrial signalling pathways or metabolic processes. Below, we expand on these, but of course it is important to note that many of these types of damage are linked and that treating one mode of mitochondrial dysfunction often has a positive impact on others.

Protecting mitochondria

Mitochondrial dysfunction in diseases can arise from sustained damage to the organelle's proteins, DNA and lipids[2,43–45]. Oxidative damage is frequently considered owing to the fairly high level of ROS production by the mitochondrial respiratory chain and the susceptibility of the organelle to oxidative damage[46,47]. Carbon stress is another disruptor of mitochondrial function that arises owing to the high levels of activated acyl-CoAs in the mitochondrial matrix that lead to non-enzymatic protein acylation, typically on lysine residues, which affects protein function and proteostasis[44,45,48].

A related common pathway of mitochondrial damage in many scenarios is the depletion of NAD^+, which can occur by activation of pathways that use up cellular and mitochondrial NAD^+ pools, such as activation of poly(ADP-ribose) polymerases (PARPs), mono-ADP ribosyl transferases and the cyclic ADP-ribose hydrolase CD38 (REFS[49–52]). One consequence of NAD^+ depletion is disruption of bioenergetic pathways. In addition, NAD^+ is required for the reversal of lysine acylation by sirtuins; hence, NAD^+ depletion also contributes to an elevation of protein lysine acylation, disrupting signalling pathways that are altered by lysine acylation and contributing to carbon stress, leading to the accumulation of damaged and misfolded proteins. Of course, many other forms of damage occur — for example, disruption due to formation of the MPTP, a large conductance channel in the inner membrane that is activated following calcium accumulation in the presence of oxidative stress, leading to mitochondrial swelling and subsequent cell death[53–55].

Defects in mitochondrial proteostasis represent another important form of mitochondrial damage that contributes to a wide range of pathologies[7,56,57]. Normally, the proteins within the mitochondria are folded correctly, and when they become damaged or misfolded they are either refolded or rapidly degraded[7,56,57]. Thus, when correctly functioning, proteostasis prevents the accumulation and aggregation of defective proteins within mitochondria, which would severely disrupt organelle function. Mitochondria face a number of challenges in maintaining proteostasis and the correct folding of proteins that are either imported into or translated within the organelle[57]. A further complication is that four of the mitochondrial oxidative phosphorylation complexes contain polypeptides encoded by both the nuclear and mitochondrial genomes; hence, the relative levels of these polypeptides have to be carefully matched to correctly assemble these complexes[57]. Finally, the mitochondrial matrix is exposed to high levels of both oxidative and carbon stress, which can damage proteins, rendering them less stable[57]. In dealing with these challenges, the mitochondria does not have a proteasome nor the same hea t shock protein complement as the cytosol. Instead, it has its own repertoire of chaperones and proteases to maintain organelle proteostasis[7,56,57]. The mitochondrial chaperones include mitochondrial heat shock protein 70 and 90 and the matrix chaperonin complex composed of mitochondrial heat shock protein 60 and 10 that help fold nascent proteins or refold misfolded ones. In addition, mitochondria contain a wide range of proteases that degrade misfolded proteins[7,56,58]. Mutations in these mitochondrial proteases lead to the accumulation of misfolded proteins and dysfunctional mitochondria in a number of diseases[58]. Furthermore, excessive oxidative damage, or protein acylation due to carbon stress, causes protein misfolding and aggregation within mitochondria. Thus factors such as replenishing

Box 2 | **Targeting small molecules to mitochondria**

The ability to selectively target compounds to mitochondria is an important development in designing drugs to affect mitochondria and thereby treat common pat hologies[2,3,7,14,41,42,283,284]. Mitochondria targeting of drugs can enhance potency, avoid side effects and speed up delivery[14,284]. There are a number of approaches to target small molecules to mitochondria. One widely used approach is to utilize the mitochondrial membrane potential ($\Delta\psi$), which drives the accumulation of lipophilic cations within mitochondria[14,41]. Lipophilic cations, notably the triphenylphosphonium (TPP) cation (but also many others), have the property of being able to pass through biological phospholipid bilayers owing to a lowering of the activation energy for movement through the bilayer[14,19,41,285]. This lowering of activation energy arises owing to distribution of the charge across a large hydrophobic surface area, either by shielding the charge in the case of TPP or by charge delocalization in the case of planar conjugated aromatic systems such as rhodamine[14,41]. The Nernst equation indicates that for every ~60 mV increase in $\Delta\psi$, the concentration of these compounds increases tenfold; hence, the compounds first concentrate in the cytosol 3–10-fold in response to the plasma membrane potential ($\Delta\psi_{plasma}$) of −30 to −60 mV and then further concentrate 200–500-fold within the mitochondrial matrix in response to the mitochondrial $\Delta\psi$ of −140 to −160 mV (REFS[14,41]). Thus, lipophilic compounds can be concentrated several thousand-fold within the mitochondrial matrix. By conjugation to a TPP, bioactive molecules can be delivered to the matrix in vivo provided they are not too polar[14,41]. Importantly, these can be delivered orally or intravenously, are rapidly taken up into many organs in vivo[285] and have been shown to be safe in the long term in human trials[22,23]. A number of different peptides can be used to target compounds to mitochondria[20,42,283,284,286]. These peptides all contain positive charges, and their uptake is assumed to be driven by the mitochondrial $\Delta\psi$, although the mechanism has been less investigated than for lipophilic cations.

the NAD^+ pool to counteract carbon stress by enhancing the activity of sirtuins, or preventing oxidative damage with antioxidants, all help maintain proteostasis. Owing to the contribution of defective proteostasis to common diseases, there is considerable interest in activating chaperones or proteases at the level of the organelle. Related to this, the mitochondrion has an unfolded protein response (mtUPR) that upregulates the expression of chaperones within the mitochondrial matrix[56,57], and enhancing the activity of the mtUPR is protective in a number of model organisms[56].

Many drugs protect the organelle directly by affecting a specific process following selective binding to a particular target site. Some drugs target matrix proteins; for example, CsA binds to the matrix protein cyclophilin D (CYD, also known as PPID) and thereby prevents cell death caused by formation of the MPTP[59]. Other compounds, such as suppressors of site I_Q electron leak (S1QELs) and suppressors of site III_{Qo} electron leak (S3QELs), bind directly to respiratory chain complexes I and III, respectively, in the mitochondrial inner membrane to inhibit ROS production[60,61]. Conversely, there are many protective molecules that act on general processes within mitochondria rather than by binding to specific targets[14,20]. These include antioxidants designed to lower mitochondrial oxidative damage[62] and molecules that enable electrons to bypass respiratory complexes in order to sustain oxidative phosphorylation despite respiratory chain damage[63]. A related intervention is the use of small-molecule uncouplers such as dinitrophenol (DNP) that decrease the protonmotive force (Δp) across the mitochondrial inner membrane, thereby making oxidative phosphorylation less efficient, which helps to burn off excess fat and to decrease mitochondrial ROS production[64,65]. The depletion of NAD^+, which can lead to both bioenergetic defects and to inappropriate protein acylation, can be counteracted by compounds such as nicotinamide (NAM), NAM riboside (NR) and NMN, which act by replenishing NAD^+ levels[50–52,66–70]. Restoring NAD^+ levels has a number of protective effects, in part by enhancing the activity of sirtuins, which act as NAD^+-dependent lysine deacylases. As protein acylation is thought to have a regulatory role in a number of metabolic processes, the positive effects of NAD^+ modulators are often ascribed to changes in regulation[66,69]. However, as lysine acylation is also a carbon stress that can lead to protein dysfunction and aggregation, it is also likely that some of the positive effects of elevating NAD^+ levels and activating sirtuins are to counteract carbon stress[44,45].

Protonmotive force (Δp). The mitochondrial respiratory chain passes electrons from NADH or flavins on to oxygen and in doing so pumps protons across the mitochondrial inner membrane, thereby establishing a Δp. The Δp is composed of a mitochondrial membrane potential ($\Delta\psi$) of ~150 mV and a pH gradient of ~0.5 pH units.

Altering mitochondrial biogenesis

Instead of directly affecting mitochondria, an important alternative therapeutic strategy is to alter organelle amount or activity by enhancing mitochondrial biogenesis[15,71–73]. Increasing mitochondrial biogenesis raises the possibility of pharmacologically increasing the mitochondrial content of the cell, the surface area of the inner membrane or the content of the oxidative phosphorylation machinery in order to increase mitochondrial ATP output, just as occurs in response to exercise[72]. This could be achieved by pharmacologically intervening at the level of the transcription factors and related regulatory proteins that control mitochondrial biogenesis[15,72,73]. There are a large number of nuclear-encoded transcription factors that control the expression of those genes involved in mitochondrial biogenesis. For example, nuclear respiratory factors NRF1 and NRF2 determine the expression of multiple nuclear genes that encode proteins targeted to mitochondria, such as DNA polymerase γ (POLG) and the DNA helicase Twinkle, which are essential for mtDNA replication[74], and transcription factor A (mitochondrial) (TFAM), which regulates expression of the 37 genes encoded by mtDNA[6,15]. There are many other transcription factors that affect mitochondrial biogenesis, such as peroxisome proliferator-activated receptors (PPARs), oestrogen-related receptors (ERRs), cAMP-responsive element-binding protein 1 (CREB1) and forkhead box protein O (FOXO)[7,15,72]; however, a detailed consideration of these is beyond the scope of this Review and is covered elsewhere[7]. Transcription factor activity is further affected by the transcriptional co-activators such as PPARγ co-activator 1α (PGC1α) and co-repressors such as nuclear receptor co-repressor 1 (NCOR1), receptor interacting protein 140 (RIP140, also known as NRIP1) and retinoblastoma proteins (pRbs), which help to coordinate organelle biogenesis and oxidative metabolism in response to changes in cell metabolic requirements (reviewed in REFS[7,15,75]). These responses are often transmitted through post-translational modifications (PTMs); for example, phosphorylation of PGC1α by the energy sensor AMP-activated protein kinase (AMPK) increases mitochondrial biogenesis in response to energy demand[13], whereas PGC1α-deacetylation by sirtuin 1 (SIRT1) enables responses to metabolic challenges[75].

A number of drugs interact with these pathways to regulate mitochondrial biogenesis by altering the activity of transcription factors[72,73]. For example, the PPARγ transcription factor can be activated directly by the anti-diabetic drugs pioglitazone and rosiglitazone as well as by the lipid metabolism modifiers bezafibrate and thiazolidinediones, which increase PGC1α expression and upregulate mitochondrial biogenesis[7,15,76,77]. Mitochondrial biogenesis can also be enhanced by drugs that indirectly alter PGC1α activity[15,75]. For example, AMPK agonists such as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) activate PGC1α, mimicking the enhancement of mitochondrial biogenesis by energy demand[78]. Another approach is to use the SIRT1 activators resveratrol and viniferin, which activate PGC1α by reversing acetylation[15]. A parallel approach to enhancing mitochondrial biogenesis is to inhibit pathways that repress mitochondrial biogenesis, such as hypoxia-inducible factor 1α (HIF1α)[79,80].

Modulating mitochondrial dynamics

Mitochondria do not exist as isolated organelles in the cell but instead undergo a continual cycle of fusing together to form larger mitochondria that then undergo fission to break up into smaller bodies[81,82]. The protein machinery that leads to these processes comprises fission proteins such as dynamin-related protein 1 (DRP1;

also known as DNM1L), whereas fusion is determined by proteins such as mitofusins (MFN1 and MFN2) on the outer membrane and optic atrophy protein 1 (OPA1) on the inner membrane[81,82]. Small molecules have been developed, such as mitochondrial division inhibitor 1 (Mdivi1), which decrease DRP1 activity and thus slow mitochondrial fission[77,83]. However, the specificity of these compounds is unclear; hence, some effects may not be due to affecting organelle division[84,85]. Modulating mitochondrial dynamics is thought to have a number of beneficial impacts on mitochondrial function and activity, although in many cases the mechanism and importance of these effects are not clear[77]. However, it is evident that one important aspect of mitochondrial dynamics is that they are intimately linked to mitochondrial quality control, discussed below.

Enhancing mitochondrial quality control

A major reason for continual mitochondrial fission and/or fusion is that it facilitates the degradation of damaged organelles by mitophagy, because small mitochondrial particles can be easily engulfed by the mitophagy machinery[71,81,82]. This requires a means of recognizing that mitochondria moving through the small particulate stage are damaged. One way in which this may be done is by their lowered Δp, which leads to accumulation of the kinase PINK on their surface. PINK in turn recruits the parkin E3 ligase, which ubiquitinylates damaged mitochondria and thereby targets them for degradation by mitophagy[86]. Although the role of this pathway in vivo is less clear[87], pathways that recognize damaged mitochondria and target them for mitophagy are a central part of mitochondrial quality control. Thus, drugs that enhance mitochondrial division may increase the clearance of defective organelles[71,81,82]. One example is AMPK activation, which can increase DRP1 recruitment to mitochondria by direct phosphorylation of the mitochondrial adaptor, mitochondrial fission factor (MFF), and thus enhance fission and subsequent autophagy of damaged mitochondria[13,88]. Increasing the removal of damaged mitochondria by mitophagy has many positive effects, such as decreasing inflammation[71]; thus, activating mitophagy is an appealing therapeutic strategy, and this has been explored with promising results using natural compounds such as urolithin A, which enhances muscle function in rodents, with possible relevance to sarcopenia[89].

There are many other ways in which mitochondrial quality control can happen at the submitochondrial level in parallel to mitophagy. Correct mitochondrial proteostasis protects against the accumulation of damaged and unfolded proteins within mitochondria[56,57]. Prevention of mitochondrial protein aggregation can be enhanced by upregulating the mtUPR response, which increases the expression of a series of chaperones within the mitochondria, and activating this response is protective in a number of model organisms[7,56]. Mitochondria can compartmentalize oxidized protein and lipid into mitochondria-derived vesicles (MDVs) that bud off from the organelle and are then targeted for degradation in lysosomes[90–92]. There are also multiple proteases, nucleases and lipases within the mitochondria that degrade damaged molecules[58]. Among these are the ATPases associated with diverse cellular activities (AAA proteases), mutations to which contribute to degenerative diseases[58]. Finally, the myriad of potentially disruptive small molecules generated within mitochondria by oxidative damage and carbon stress can be conjugated to glutathione by glutathione *S*-transferases and the resulting conjugate exported by ATP-binding cassette (ABC) proteins[93]. Thus, enhancing the clearance of damaged mitochondria and the organelle's components is a promising therapeutic strategy for future development.

Harnessing mitochondria to kill cells

The central role of mitochondria in cell death by apoptosis or necrosis makes them a good target when aiming to kill a particular cell[55,94], such as a cancer cell or a protozoan parasite. Although it is easy to kill cells non-selectively by targeting mitochondria, the challenge is to do so selectively. Mitocho ndria are similar in most cells; consequently, any small difference in the mitochondrial function of target cells makes an appealing target[95]. Therefore, using mitochondria to kill cancer cells necessitates focusing on how they differ from non-transformed cells or selectively activating a toxic pro-drug within the target cell. For example, many cancer cells have ineffective mitochondrial apoptosis that can be re-activated[96]. Another approach is to deplete antioxidant defences[97], or to increase mitochondrial ROS production[98], and combine these cell stressors with another cancer drug to induce synthetic lethality[97].

Altering mitochondrial signalling

A rapidly expanding area of mitochondrial biology is the role of the organelles as signalling hubs that respond to and influence processes throughout the cell[6,99,100]. The signals that emanate from mitochondria to the rest of the cell include changes in the ATP:ADP ratio, Ca^{2+}, NAD^+, metabolites and ROS, but our understanding of their nature, targets and physiological roles is still developing[99]. Redox signalling by the production of ROS (such as hydrogen peroxide) that modify protein activity through the reversible oxidation of redox-sensitive cysteine residues has been a long-standing focus[99,101,102]. More recently, there has been considerable interest in how citric acid cycle (CAC) metabolites are transmitted back and forth between the mitochondrion and the cytosol as a way of regulating cell function and fate[103,104]. For example, histone acetylation is sensitive to acetyl-CoA levels that are determined by citrate export from the mitochondria[105]. Furthermore, there are numerous 2-oxoglutarate-dependent dioxygenases, including prolyl hydroxylases in the HIF1α oxygen-sensing pathway, ten-eleven translocation (TET) DNA demethylase and the histone lysine demethylase Jumonji C[106]. These enzymes utilize 2-oxoglutarate as a substrate and are inhibited by succinate, hence providing a link between mitochondrial CAC activity and the regulation of oxygen sensing and the formation of epigenetic marks on the genome[106,107]. Thus, the manipulation of CAC metabolite transfer between the mitochondrial matrix and the rest of the cell may be a useful therapeutic approach[108].

Citric acid cycle
(CAC). The CAC takes acetyl-CoA generated from the pyruvate produced by glycolysis to fuse with oxaloacetate to form citrate. The citrate is then broken down to release carbon dioxide while providing electrons to the respiratory chain and regenerating oxaloacetate to keep the CAC turning.

Treating pathologies via mitochondria

The general principles of how and why to treat mitochondria in common pathologies have been outlined above. Here, we consider some concrete examples of the common pathologies ischaemia–reperfusion (IR) injury, inflammation, the metabolic syndrome, neurodegeneration, HF and protozoal infection in which therapies focused on mitochondria are likely to be effective (TABLE 1); discuss the approaches used; and suggest future directions. Mitochondria are also proving to be an interesting therapeutic target in cancer therapies; however, the diversity of this field puts it beyond the scope of this Review, and a few key points are considered in BOX 3. Mitochondria are also emerging as potential targets in many other common pathologies, including muscular dystrophies[69], sarcopenia[109], lung diseases[110] and colitis[111], and the reader is referred to the cited papers and reviews for more detail.

IR injury

Ischaemia ensues when the blood flow to an organ is disrupted, depriving it of oxygen and its supply of external metabolites while also causing a build-up of metabolic products such as lactate and succinate[112–115] (FIG. 2). The lack of oxygen and respiratory substrates stops oxidative phosphorylation, causing the ATP:ADP ratio to fall, which in turn leads to adenine nucleotide breakdown[116]. The obvious remedy for ischaemia is to restore blood flow as quickly as possible to the affected tissue. For example, the standard of care for the most damaging form of heart attack, ST-elevation myocardial infarction (STEMI), is to remove the blockage from the cardiac artery by primary percutaneous coronary intervention (PPCI)[30]. Despite prompt reperfusion by PPCI, extensive tissue damage known as IR injury is still a major cause of morbidity and mortality[117]; thus, a major unmet need is a treatment that can be administered to the patient at the same time as PPCI[30,117]. Similarly, in ischaemic stroke, the standard of care is to restore blood flow through thrombolysis by infusion of tissue plasminogen activator (TPA)[118] or by angiographic revascularization[119]. These interventions rapidly restore blood flow, but paradoxically the restoration of oxygenated blood to the ischaemic tissue itself leads to IR injury[112–115,120]. IR injury is a key driver of pathology in heart attack and stroke[112,114,115] but also in many other pathologies, including acute kidney injury[121], muscle injury[122] and the organ damage associated with organ transplantation and elective surgery[123]. Although there has been considerable clinical progress in minimizing the duration of ischaemia in

Table 1 | **Therapeutic strategies targeted to mitochondria in common pathologies**

Agent	Mode of action	Disease and/or effect	Trial and/or animal model	Refs or ClinicalTrials.gov identifier
Protection				
Cyclosporin A	Block MPTP	Heart attack	CIRCUS phase III	[138]
			CYCLE phase II	[139]
CoQ_{10}	Antioxidant	Heart failure	Q-SYMBIO phase II	[235]
MitoQ	Mitochondria-targeted antioxidant	Parkinson disease	PROTECT phase II	[208]
		Chronic kidney disease	Mitochondrial oxidative stress and vascular health in chronic kidney disease phase IV	NCT02364648
		Hepatitis C	Phase II	NCT00433108, [278]
MTP-131 (Bendavia/SS31)	Unknown	Heart attack	EMBRACE STEMI phase II	[143]
		Skeletal muscle mitochondrial dysfunction in the elderly	MOTION phase II	NCT02245620
Biogenesis				
AICAR	Activates AMPK, which then acts on PGC1α	Oxidative phosphorylation defect	Mouse model of myopathy	[68]
Dynamics				
Mdivi1	DRP1	Slowed mitochondrial fission	Mouse model of excitotoxicity	[73] but see [74,75]
Quality control				
Urolithin A	Enhanced mitophagy	Muscle function	Mouse models of ageing-associated skeletal muscle decline	[79]
Signalling				
NMN	Increase NAD^+ pools	Activate mitochondrial unfolded protein response	Mouse model of fatty liver disease	[44]
		Enhance multiple NAD^+ dependent pathways	Mouse model of Alzheimer disease	[57]

AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AMPK, AMP-activated protein kinase; CoQ_{10}, coenzyme Q_{10}; DRP1, dynamin-related protein 1; Mdivi1, mitochondrial division inhibitor 1; MPTP, mitochondrial permeability transition pore; NMN, nicotinamide mononucleotide; PGC1α, peroxisome proliferator-activated receptor-γ co-activator 1α.

Box 3 | **Mitochondria in cancer therapies**

It is now clear that many cancer cells reprogramme their metabolism and mitochondrial function to provide the building blocks to generate lipids, proteins and nucleic acids and to sustain mitotic signals to enable cell proliferation[287–289]. Consequently, changes in mitochondrial metabolism and redox status are now considered hallmarks of cancer[287,288,290]. The metabolic reprogramming of mitochondria in cancer was first noted by Warburg, who found that cancer cells converted large amounts of glucose to lactate even in the presence of oxygen, a phenomenon that was later defined as aerobic glycolysis[291,292]. Initially, it was thought that this metabolic feature of cancer arose from mitochondrial dysfunction or damage; however, it is now clear that aerobic glycolysis is an inherent property of cancer and that functional mitochondria are essential for cancer cells to proliferate[287,293,294]. Further properties of many cancer cells are enhanced mitochondrial reactive oxygen species (ROS) production and a redox imbalance that is thought to stimulate cell proliferation and inhibit growth suppression[287,295,296]. Although this summary is inevitably an oversimplification, it shows why targeting mitochondrial metabolism is a promising approach to kill cancer cells[287,290,297].

A further aspect of mitochondria in most cancer cells is their higher protonmotive force (Δp) than in non-transformed cells[298–302]. One factor contributing to this may be that the high flux of ATP production by glycolysis decreases Δp utilization for ATP synthesis by oxidative phosphorylation[287,293,294]. Irrespective of the underpinning reasons, the elevated mitochondrial Δp in cancer cells, usually manifesting as an elevated Δψ, is a well-established attribute of many cancer cells[303–305] and can be used to selectively enhance drug uptake into the mitochondria of cancer cells compared with untransformed cells[302–305].

Many of these properties of mitochondria in cancer cells can be used to enhance cell killing. For example, oxidative phosphorylation is required for cancer cell survival and growth[287,293,294,306]; hence, selectively disrupting this process in tumour mitochondria without overt toxicity to other cells is an appealing therapeutic possibility. Although there are considerable uncertainties and variations, many cancer cells seem to have enhanced mitochondrial ROS production, which is thought to act as a mitogenic signal[287,295,296,307–310]. This putative enhancement of mitochondrial ROS production reveals two therapeutic strategies[307]. The first strategy is to disrupt mitogenic ROS signalling from mitochondria, as has been shown in animal models using mitochondria-targeted antioxidants to inhibit cell proliferation and metastasis[287,295,311]. The other therapeutic strategy utilizes the fact that cancer cells often upregulate their antioxidant defences, possibly to cope with the higher levels of redox stress associated with mitochondrial mitotic signals[287,296]. The greater oxidative stress in some cancer cells makes them more susceptible to disrupting mitochondrial antioxidant defences than non-transformed cells[296,310,312]. Many cancer cells evade death owing to defective induction of the mitochondrial apoptotic pathway (for example, due to overexpression of the anti-apoptotic protein B cell lymphoma 2 (BCL-2))[96,313]. The point of no return for mitochondrial apoptosis is induction of mitochondrial outer membrane permeabilization (MOMP) and the subsequent release of pro-apoptotic factors such as cytochrome *c* into the cytosol[314,315]. Pro-apoptotic proteins, such as BCL-2-associated X (BAX) and BCL-2 homologous antagonist/killer (BAK), form the MOMP pore, and these proteins are normally held in check by anti-apoptotic proteins of the BCL-2 family[314,315]. The balance between these anti-apoptotic and pro-apoptotic proteins is determined by the BH3-only pro-apoptotic proteins, such as truncated BID, which bind to anti-apoptotic members of the BCL-2 family, leading to MOMP[316]. Thus, BH3 mimetic drugs such as venetoclax have been developed to counteract the suppression of apoptosis in cancer cells by excess BCL-2 anti-apoptotic members and thereby use mitochondria to kill cancer cells[96,309,313,315].

In summary, the role of mitochondria in several facets of cancer progression, coupled with the possibility of enhanced selectivity in targeting mitochondria within cancer cells, suggests multiple novel therapeutic approaches.

many pathologies, there is now increasing interest in developing therapies that decrease the inevitable IR injury that occurs on reperfusion of ischaemic tissues[115].

Mitochondrial ROS production in IR injury. The initiating factor of IR injury is a burst of the ROS superoxide from the mitochondrial respiratory chain upon reperfusion that initiates a cascade of tissue damage[114,115]. This process had long been tacitly assumed to be a random consequence of the reperfusion of ischaemic tissue; however, recent work suggests that IR injury occurs as a result of specific processes and is not just a catastrophic breakdown of cell function[114,124] (FIG. 2). During ischaemia, the CAC metabolite succinate builds up dramatically, then upon reperfusion the accumulated succinate is rapidly oxidized, driving superoxide production at complex I by reverse electron transport (RET)[114] (FIG. 2). The superoxide production results in oxidative damage that disrupts mitochondrial function and, in conjunction with calcium accumulation within mitochondria during ischaemia, leads to induction of the MPTP[125–127]. The cell death and organ dysfunction caused by induction of the MPTP lead to the release of mitochondrial and cell contents, resulting in the activation of an inflammatory response that can further damage tissue and will ultimately give rise to tissue scarring and remodelling[128]. Whether or not this model of IR injury stands the test of time, it does seem to account for much of the confusing literature in the field and can be used to generate rational therapies, furthermore it provides a useful framework for discussing mitochondrial therapies for IR injury[114] (FIG. 2).

Metabolic changes in IR injury. Succinate accumulation during ischaemia and its oxidation during reperfusion are key drivers of IR injury[129–131]. Malonate is a potent inhibitor of succinate dehydrogenase (SDH), and its cell-permeable form dimethyl malonate (DMM)

Reverse electron transport (RET). Complex I in the mitochondrial respiratory chain can produce superoxide by RET. This occurs when the protonmotive force (Δp) is high and the ratio of ubiquinol (QH_2) to ubiquinone (Q) in the CoQ pool is high, causing electrons to flow backwards through complex I.

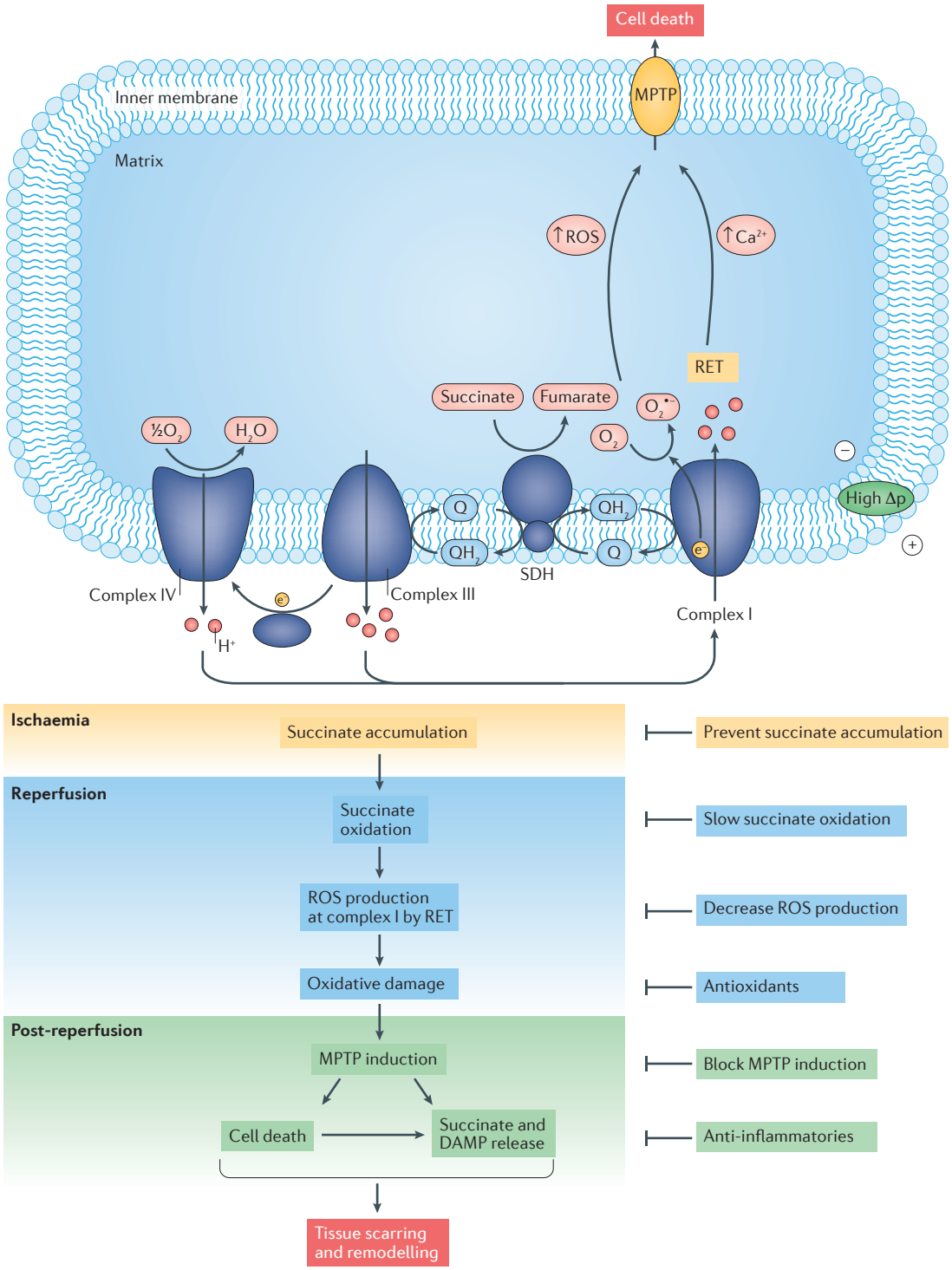

Figure 2 | **Mitochondria as a therapeutic target in ischaemia–reperfusion injury.** Ischaemia arises when blood flow to an organ is restricted. This restriction causes the accumulation of metabolites such as lactate, succinate and depletion of ATP as well as disruption to calcium homeostasis. When blood flow is restored, there is rapid oxidation of the accumulated succinate that drives reactive oxygen species (ROS) production at complex I by reverse electron transport (RET). This induces oxidative damage and in conjunction with an accumulation of calcium leads to induction of the mitochondrial permeability transition pore (MPTP), resulting in cell death. This model of ischaemia–reperfusion (IR) injury applies to IR injury in many contexts that leads to tissue damage, opening up rational mitochondrial interventions. Potential therapeutic strategies include preventing the accumulation of succinate, preventing the oxidation of succinate upon reperfusion, preventing ROS production by complex I, blocking the downstream effects of ROS or preventing induction of the MPTP. In addition, the release of succinate and mitochondrial damage-associated molecular patterns (DAMPs) into the circulation act as pro-inflammatory signals that will contribute to tissue damage following IR injury. Δp, protonmotive force; e^-, electron; Q, ubiquinone; Q; QH_2, ubiquinol; SDH, succinate dehydrogenase.

decreases both succinate accumulation during ischaemia and its oxidation upon reperfusion[129]. Furthermore, addition of malonate upon reperfusion is also protective[130,131]. In addition, some succinate is released from the ischaemic tissue into the circulation upon reperfusion[132] and can activate the pro-inflammatory succinate receptor (SUCNR1), which is expressed in immune cells, thereby stimulating inflammatory damage[133–135]. These findings suggest that inhibitors of succinate accumulation during ischaemia, and of its oxidation and release during reperfusion, are promising therapeutic agents[136].

Complex I as a target in IR injury. Succinate oxidation upon reperfusion generates ROS at complex I by RET, and this ROS production can be blocked with the complex I inhibitors rotenone[137], with S1QELs[61] or by the mild uncoupling of mitochondria in order to lower Δp, a driving force for RET[138]. These findings suggest that inhibiting RET at complex I transiently during reperfusion blocks the ROS burst, with complex I activity returning to normal when the succinate accumulated during ischaemia has been oxidized. For example, inhibiting complex I temporarily during reperfusion with the mitochondria-targeted *S*-nitrosating agent MitoSNO decreases cardiac IR injury in mice[139–141]. The reversible inhibition of complex I is brought about by *S*-nitrosating a particular cysteine residue that is exposed only during ischaemia when complex I undergoes a conformational shift to a deactive state[141]. *S*-Nitrosation temporarily locks complex I in the deactive state, preventing RET upon reperfusion, but as the modification is reversible, the activity of complex I is restored to normal a few minutes after reperfusion[141]. It is likely that many other agents that protect against IR injury, such as hydrogen sulfide[142,143], act in a similar way to decrease ROS production upon reperfusion[114].

The next point of intervention is to protect mitochondria from oxidative damage during IR injury[144]. Exogenous antioxidants are protective against IR injury[144], and mitochondria-targeted antioxidants have also shown protection against cardiac[145,146] and kidney[147] IR injury. However, a limitation is that the antioxidant was administered before IR injury, and it may not be taken up rapidly enough to be effective when added upon reperfusion to treat heart attack or stroke. Even so, mitochondria-targeted antioxidants may be useful for situations in which IR injury is predictable, such as elective surgery or organ transplantation.

The MPTP in IR injury. Blocking MPTP induction is the next point to protect mitochondria during IR injury[125–127]. Although the nature of the MPTP is still not definitively established, it is clear that the mitochondrial *cis–trans* prolyl isomerase CYD is required for induction of the MPTP under pathological conditions[125–127].

The MPTP can be blocked by infusion of the CYD inhibitor CsA at reperfusion[148], immediately suggesting a drug treatment for IR injury in humans. When CsA was administered at the same time as PPCI in a phase II trial of patients with STEMI, it showed promising results[149]. However, when extended to phase III in the CIRCUS[26] and CYCLE trials[27], it was unsuccessful. The drug TRO40303, which binds to mitochondrial outer membrane translocator protein (TSPO) and is thereby thought to inhibit the MPTP, was also unsuccessful against STEMI in the MITOCARE study[150,151]. The mitochondria-targeted peptide Bendavia (SS31) showed promising results against IR injury in animal studies[152], although its mechanism of action is unclear, but it too was unsuccessful when administered during PPCI to patients with STEMI in the EMBRACE STEMI study[24].

Translation of IR therapies to the clinic. Although treatment of IR injury with mitochondrial therapeutics is well justified by animal studies, when it was attempted in a well-defined clinical scenario — PPCI of patients with STEMI — the outcome has thus far been disappointing[153]. There are several factors contributing to this[30]: the animals used were young and healthy, lacking the comorbidities of old and unhealthy patients; patients were on multiple medications that may act on the same pathways as the drugs being assessed, offering little scope for further protection; the duration of ischaemia before treatment may have been too short, therefore the tissue will fully recover anyway, or too long, making salvage of the organ impossible; and the uptake of drugs such as CsA into mitochondria may have been too slow to stop the cell damage, hence the need to administer the drugs very rapidly to the tissue. For many of the drugs investigated thus far, administration must occur at the time of or very shortly after the onset of reperfusion. Clinical trials should be designed more carefully to address these pitfalls[117,154]. Despite the disappointments, we believe that therapies targeted at preventing ROS production upon reperfusion[129–131,141] have potential in humans, either alone or as part of a combination therapy targeted to multiple nodes of mitochondrial damage during IR injury.

In summary, preventing mitochondrial damage during IR injury remains a promising treatment strategy, and the hope is that treatments focused on mitochondria will lead to new therapies for a range of pathologies. The common mitochondrial pathway for IR injury suggests that many of the therapies under development can be applied to other clinical situations when IR injury arises, such as elective surgery, organ transplantation, acute trauma or stroke. Using mitochondrial therapies to treat stroke is particularly appealing as such treatments can be given safely to patients before a brain scan in hospital, which is mandatory before thrombolysis or thrombectomy to determine whether it is an ischaemic or haemorrhagic stroke. IR injury in stroke is far less investigated than in myocardial infarction, and the translation of protective strategies has been frustratingly slow. Furthermore, although mortality and morbidity for myocardial infarction have declined in recent years owing to early reperfusion, this is not the case for stroke, therefore focusing on mitochondria may help address this unmet need.

Pathological inflammation

Inappropriate activation of inflammation contributes to the aetiology of many common disorders, ranging from the acute inflammatory response in sepsis to the chronic autoimmune diseases multiple sclerosis, lupus and rheumatoid arthritis[4,120,155]. Mitochondria contribute to the tissue damage that leads to inflammation and also play a role as signalling hubs in key immune cells such as T cells and macrophages[4,156]. Resting monocytes and/or macrophages and lymphocytes rely on oxidative phosphorylation, but following immune activation, their metabolism is reprogrammed to aerobic glycolysis and glutaminolysis to support cell proliferation[4,110,157]. Thus, new therapies targeted to mitochondria are a promising way to intervene in disorders associated with inflammation[4,110,157].

Mitochondria play an important role in the activation of innate immune signalling[29,110]. Owing to their endosymbiotic origin from α-proteobacteria, mitochondria can be considered as ancient 'enemies within' that reveal themselves as such only when their contents are released[29]. These mitochondrial components are then recognized as damage-associated molecular patterns (DAMPs) by the innate immune system, akin to the pathogen-associated molecular patterns (PAMPs) that activate the innate immune system in response to bacterial or viral infections[29]. DAMPs released by mitochondria include *N*-formyl peptides, which are made during mitochondrial (and bacterial) protein synthesis, but not by eukaryotic cytoplasmic ribosomes[158]. Another important DAMP is mtDNA, on which CpG islands are hypomethylated compared with those on eukaryotic nuclear DNA, but again is similar to bacterial and viral DNA[158]. Mitochondrial DAMPs also provide a signal to initiate repair following tissue injury by binding to receptors of the innate immune system[29]. These mitochondrial DAMPs can act both within the cell or following their release into the circulation[159]. In many disorders, this immune activation by tissue damage contributes to the pathology. Hence, many approaches that protect against mitochondrial damage, such as antioxidants or CsA, exert some of their clinical benefit by decreasing immune activation through limiting the release of mitochondrial DAMPs[114]. Mitochondria contribute to the initiation of inflammatory signalling pathways within cells in a number of ways. One way is through the assembly of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome on the surface of the mitochondrial outer membrane in response to mitochondrial damage and elevated ROS levels, leading to the maturation of pro-inflammatory cytokines such as IL-1β and IL-18 (REFS[4,160,161]). These inflammatory pathways can also be activated in response to viral infection through the mitochondrial antiviral signalling pathway on the mitochondrial outer membrane[29,162]. Thus, mitochondria are involved in the activation of innate immune signalling in a number of ways.

Mitochondria also play an important role in the adaptive immune response; for example, $CD4^+$ helper T cells and cytotoxic $CD8^+$ T cells reprogramme their metabolism away from oxidative phosphorylation to aerobic glycolysis and glutaminolysis, which supports the elevated mitochondrial ROS production and cytokine production that enables subsequent T cell proliferation and is sustained through epigenetic changes[4,156,163]. Mitochondrial metabolism in macrophages is also reprogrammed in a similar way when they shift from the anti-inflammatory M2 phenotype to the pro-inflammatory M1 phenotype in response to infection and tissue damage, subsequently returning to the M2 phenotype to help resolve the inflammation[4]. The shift of macrophages to the M1 phenotype is associated with elevated succinate generation by mitochondria, which stabilizes HIF1α and generates mitochondrial ROS by RET at complex I (REFS[104,164]). Together, these signals activate downstream transcriptional pathways that sustain macrophage proliferation and cytokine production in response to infection or tissue damage[104,164]. In addition, upon its release from cells into the plasma, succinate acts as a pro-inflammatory signal by binding to SUCNR1, a G protein-coupled receptor on the surface of cells in the retina, kidney and immune system that responds to extracellular succinate to activate a pro-inflammatory signalling pathway[135,165].

In summary, mitochondrial damage, elevated ROS production and succinate generation are frequently associated with inflammation. Therefore, pharmacological interventions that decrease mitochondrial damage or alter signalling pathways by decreasing mitochondrial ROS production and succinate generation and/or oxidation may prevent an excessive immune response. Supporting this, animal models of sepsis have shown that mitochondria-targeted antioxidants[166,167] and inhibitors of succinate oxidation[164] are protective. Furthermore, mitochondria-targeted antioxidants have also shown efficacy in animal models of autoimmune diseases such as multiple sclerosis and tumour necrosis factor receptor periodic disease (TRAPs)[157,168]. Although these approaches have yet to be translated to the clinic, they suggest that therapies focused on mitochondria are an emerging way of limiting pathological inflammation.

The metabolic syndr ome

The metabolic syndrome comprises a cluster of symptoms including central obesity, insulin resistance, elevated blood pressure and raised levels of circulating glucose, triglycerides and cholesterol[169,170]. The metabolic syndrome is at epidemic levels in both the developed and developing world, greatly increasing the risk of pathologies including type 2 diabetes, heart attack, stroke, fatty liver and HF, with considerable economic, social and medical consequences[169]. Although lifestyle changes could address many cases of the metabolic syndrome, there remains a large unmet need for better treatments to, ideally, address the underlying pathology, or at least ameliorate the symptoms. As overnutrition and lack of physical activity are frequently associated with the metabolic syndrome, it is unsurprising that mitochondrial dysfunction is central to its development[170,171].

Obesity. Central obesity is a key component of the metabolic syndrome, and decreasing obesity by bariatric surgery is an effective treatment for the metabolic syndrome[172]; hence, reducing obesity pharmacologically is appealing medically and aesthetically[65]. An obvious way to decrease adipose tissue is to burn off stored fat as heat[173]. Uncoupling protein 1 (UCP1) in brown adipose tissue releases the chemical potential energy stored in fat as heat rather than as a high ATP:ADP ratio[65]. This occurs because UCP1 facilitates increased proton movement through the mitochondrial inner membrane, thereby making oxidative phosphorylation less efficient[173]. Small-molecule protonophoric uncouplers such as DNP are very effective at decreasing obesity in humans in this way[65,174]. However, in 1938, the FDA banned the use of DNP as a slimming agent because its narrow therapeutic window led to cases of fatal hyperthermia[64,65,174]. Thus, a safe mitochondrial protonophore with a far wider therapeutic index than DNP has considerable appeal for treating the metabolic syndrome[65]. One promising approach is through a DNP methyl ether that is preferentially metabolized to DNP by cytochrome P450 in the liver, selectively releasing DNP and decreasing fatty liver disease, hyperlipidaemia and insulin resistance with far less toxicity than DNP[174–176]. An alternative approach is to use a self-limiting protonophore that would induce proton leak only in mitochondria with a high Δp but that would then inactivate itself once the Δp decreased systems[177,178]. It may also be possible to enhance uncoupling by activating endogenous mitochondrial proteins to dissipate the Δp (for example, by cysteine modification of UCP1 in brown adipose tissue)[179]. Mitochondrial oxidative phosphorylation could also be made less efficient by allowing electrons to bypass proton pumping respiratory chain complexes, as is achieved by the direct transfer of electrons from the coenzyme Q (CoQ) pool to oxygen using the alternative oxidase (AOX) in plants and protozoans[39]. However, replicating this process with small molecules without generating ROS is a major challenge. Oxidative phosphorylation can also be rendered less efficient by degrading ATP non-productively in a futile cycle, which is how shivering generates heat. There are interesting recent reports that creatine phosphate can be hydrolysed in this way[180,181], but whether this process can be pharmacologically manipulated is not yet known. It may also be possible to enhance ATP hydrolysis more directly, the potential of which is illustrated by arsenate, which substitutes for phosphate during mitochondrial ATP synthesis to form ADP–arsenate, which hydrolyses spontaneously[182,183]. In summary, decreasing mitochondrial efficiency is an appealing strategy to treat the metabolic syndrome, which has been tainted by its past association with the unregulated use of DNP as a slimming pill[65]. Promising new approaches with enhanced selectivity are emerging; therefore, it should be possible to gradually decrease obesity without dangerously disrupting energy metabolism[175,176].

Insulin resistance. Another hallmark of the metabolic syndrome is insulin resistance, whereby tissues, notably skeletal muscle, are less effective at taking up glucose in response to insulin and liver glucose output is not shut down[16]. Metformin is a widely used drug for type 2 diabetes that inhibits complex I, elevates the ADP:ATP ratio and thereby activates liver AMPK to slow liver gluconeogenesis[184]. Mitochondrial dysfunction has long been associated with insulin resistance; however, the mechanism is not known, and it is unclear whether defective mitochondrial function is a cause or a consequence of insulin resistance[171]. Even so, there is considerable circumstantial evidence linking elevated mitochondrial ROS production and organelle dysfunction with insulin resistance, as well as with ectopic lipid accumulation and chronic inflammation[185,186,171]. This is supported by studies in which decreasing mitochondrial ROS production and oxidative damage by the use of mitochondria-targeted antioxidants restored insulin sensitivity and attenuated associated factors such as hyperlipidaemia[187–189]. Chronically elevated blood glucose leads to a range of complications in both type 1 and 2 diabetes, including microvascular disease damaging small blood vessels that particularly affects the retina, peripheral neurons and the kidney[16,190]. Increased mitochondrial ROS production is thought to be one consequence of the elevated glucose[190,191]. Consistent with this, mitochondria-targeted antioxidants have shown promise in decreasing diabetic complications[16]. Furthermore, in mouse models of type 2 diabetes, there is depletion of the NAD^+ pool, and ameliorating this with NMN has shown efficacy[70], suggesting that the bioenergetic and proteostatic defects associated with NAD^+ depletion contribute to the metabolic syndrome and that restoring the NAD^+ pool is a promising therapeutic approach.

Hypertension. Mitochondrial oxidative damage and elevated production of superoxide in endothelial cells is a contributing factor to the elevated blood pressure seen in the metabolic syndrome[192]. This elevation in blood pressure is thought to occur owing to mitochondrial superoxide reacting with and thus sequestering the vasorelaxant NO[193]. In addition, the elevated production of ROS leads to oxidative damage to extracellular elastase[192], which also contributes to hypertension. These findings suggest that decreasing mitochondrial ROS production and preventing the associated oxidative damage is a potential therapy for hypertension. Supporting this view, the administration of mitochondria-targeted antioxidants to rodents was shown to lower blood pressure[193–195]. These studies also indicated that the positive effects on hypertension were associated with less mitochondrial ROS production, consistent with a key role for mitochondrial oxidative stress in hypertension. One limitation to these studies was that the mitochondria-targeted antioxidants were given while the hypertension developed in the animals, rather than once hypertension was established. In a study with old (~27-month-old) mice, 4 weeks of MitoQ treatment reversed established aortic stiffness[196]. This study was then extended to older human volunteers (60–79 years of age) with impaired endothelial function indicated by impaired brachial artery flow-mediated dilation[197]. In this placebo-controlled crossover design study, it was found that 6 weeks of oral supplementation with MitoQ improved

brachial artery flow-mediated dilation[197]. These studies suggest that mitochondria are a promising therapeutic target for hypertension.

Nonalcoholic fatty liver disease. Nonalcoholic fatty liver disease (NAFLD) is frequently associated with the metabolic syndrome, both as a consequence and as a contributor to the pathology[198]. NAFLD comprises a range of pathologies, beginning with fatty liver, or steatosis, and progressing to nonalcoholic steatohepatitis (NASH), which in turn often leads to liver fibrosis and finally to cirrhosis[198,199]. NAFLD is the most common form of chronic liver disease in the western world and is strongly associated with obesity. Treatment options for NAFLD are limited, with liver transplantation being the only possibility for cirrhosis[198]. The accumulation of fat in the liver is the key driver of NAFLD, and this can be addressed directly by enhancing mitochondrial fat oxidation by inducing selective mitochondrial uncoupling in the liver using DNP derivatives[175,199]. In addition, mitochondrial damage is intimately linked to the development of NAFLD, with elevated oxidative stress and NAD^+ depletion[51,200]. Consequently, in animal models of NAFLD, there have been demonstrations of efficacy with mitochondria-targeted antioxidants such as MitoQ[187–189] and with NMN, which replenished the NAD^+ pool[51]. Thus, treatments aimed at enhancing mitochondrial fat oxidation or at protecting mitochondria against damage are both appealing strategies for treating NAFLD.

Neurodegenerative diseases

Most current treatments for neurodegenerative disorders are aimed at alleviating symptoms; therefore, therapies that slow or stop the progression of neurodegeneration are desperately needed[15,120,201–205]. However, the search for disease-modifying treatments is hampered by our limited knowledge of neuronal cell death mechanisms in these disorders, even when the gene responsible is established, as in Huntington disease (HD) and familial Parkinson disease (PD). Even so, there is a long-standing and robust consensus that mitochondrial dysfunction is strongly associated with a wide range of neurodegenerative diseases, including PD, Alzheimer disease (AD), amyotrophic lateral sclerosis, HD and Friedreich ataxia[77,120,202,203]. This association between mitochondrial dysfunction and neurodegeneration is supported by in vitro studies, genetic and toxin animal models, post-mortem human brain tissue and human genetic studies[120,202–204,206,207]. Many types of mitochondrial dysfunction have been associated with neurodegeneration, including oxidative damage, defective ATP synthesis, NAD^+ depletion, limited mitochondrial dynamics and quality control, disrupted calcium homeostasis and the association of protein or peptide aggregates with mitochondria[120,202,203]. Thus, there is a clear consensus that mitochondrial dysfunction is closely associated with neurodegeneration, but whether organelle dysfunction is a cause, a consequence or part of a self-sustaining vicious cycle of damage is difficult to deconvolute. However, resolving these issues is not essential for drug development, as therapies that protect mitochondria work in genetic and toxin animal models of neurodegenerative disorders[31,202,206,208]. Among the treatments that are protective against mitochondrial damage and have shown efficacy in animals are antioxidants such as CoQ_{10}, mitochondria-targeted antioxidants such as MitoQ and mitochondria-targeted peptides such as Bendavia (SS31)[209,210]. Therapies that enhance mitochondrial biogenesis by increasing the activity of transcription factors such as PGC1α and NRF2, or of AMPK, are also effective in animals models[203]. In addition, replenishing NAD^+ pools with molecules such as NMN[67] or altering mitochondrial dynamics[211,212] have also shown benefit in animal models. Of particular interest is the potential to use these interventions to address defects in mitochondrial proteostasis, which contribute to a range of neurodegenerative diseases[68,213].

Despite these promising data in animals, the translation of mitochondrial therapies to the clinic has been disappointing[31]. For example, creatine, CoQ_{10} and NRF2 were ineffective in PD or AD[77,202,214,215], and the mitochondria-targeted antioxidant MitoQ showed no effect in PD[23]. Why the lack of success? In our view, the extensive animal and human data indicate that targeting mitochondria is a good strategy that should slow the progression of neurodegenerative diseases. A likely factor contributing to the lack of success to date is that by the time a patient with a neurodegenerative disease is recruited to a clinical trial, the pathology is already too firmly established to be treated. By contrast, in many animal studies, therapies are given before the onset of clinically evident symptoms. Related to this, many neurodegenerative disease processes may constitute a vicious spiral, such that once the cell damage is initiated, other factors, such as inflammation and vascular damage, contribute to a feedforward spiral of death. Thus, by the time the disease is symptomatic, it may already be too late to intervene at the level of the mitochondria.

Possible ways to improve the translation of mitochondrial drugs to the clinic are to screen compounds in animal models after neurological symptoms are well established, to determine whether the drug can slow progression before moving to human trials. A corollary is the urgent need for early diagnosis in as-yet asymptomatic patients so that clinical trials can be initiated well before irreversible damage has occurred. In the absence of presymptomatic diagnosis, we can focus trials on patients with a strong likelihood of developing a neurodegenerative disease, such as those with HD[216], Down syndrome[217], familial forms of PD[218] or subjects predisposed to AD owing to the presence of the homozygous ε4 allele of apolipoprotein E[219]. We remain optimistic about the potential of mitochondrial therapies for the treatment of neurodegenerative diseases, particularly those designed to prevent mitochondrial damage, increase organelle biogenesis or enhance mitochondrial quality control. However, these developments require advances in early diagnosis, the development of clinically relevant biomarkers and improved trial design to enable the faster evaluation of compounds in the clinic.

Retinal dysfunction. An important subset of neurodegenerative diseases that have a strong mitochondrial component are those due to retinal defects[220,221]. Damage or loss of retinal photoreceptor cells (RPCs) is the most common cause of sight loss in the western world, with the most prevalent form being age-related macular degeneration (AMD)[220,221]. The most common, 'dry' form of AMD is caused by loss of retinal pigment epithelia (RPE) cells that sustain photoreceptor cells[221]. In addition, there are a number of inherited conditions that predispose to photoreceptor loss, the most common of which is retinitis pigmentosa (RP)[220]. The RPCs, RPE and Müller glial cells all contain many mitochondria, making the retina one of the most oxidatively active tissues[222,223]. In addition, the retina is exposed to high levels of oxidative stress due to light exposure[224]. The dependence on oxidative phosphorylation and high levels of oxidative stress make the retina very susceptible to mitochondrial dysfunction and suggests that treatments focused on this organelle are beneficial[222]. This is supported by findings in animal models showing that RPC death is associated with NAD^+ depletion, leading to decreased SIRT3 activity, and that NAD^+ repletion with NMN decreases this cell loss[52]. Furthermore, treatment with a mitochondria-targeted antioxidant in an animal model of AMD decreased oxidative stress and inflammation[225]. Although a number of challenges remain, such as the selective delivery of molecules to the retina, preliminary data and the importance of mitochondria in retinal pathologies suggest that this is an important area for future development.

Heart failure

There are multiple causes and variants of chronic HF[226], but in all cases it leads to progressive cardiac dysfunction and inadequate blood pumping[227–230]. Current treatments for HF include beta-blockers, angiotensin-converting enzyme inhibitors, vasorelaxants and diuretics, which predominantly act by lowering the workload on the failing heart[231,232]. Drugs capable of improving heart contractility and blood pumping in HF without the adverse effects associated with positive inotropic therapy are needed[32].

The energy-demanding blood pumping by the heart relies on mitochondrial ATP production to both drive cardiomyocyte contraction and redistribute the calcium released to initiate this process[233]. Metabolic supply and demand are closely matched so that the heart can adapt rapidly to the fivefold to sixfold increase in workload required for maximum physical activity[233]. Hence, it is unsurprising that mitochondrial dysfunction is a key component of HF[227–230,234]. This is illustrated by the metabolic remodelling in the failing heart, which shifts from fatty acid oxidation towards glucose utilization because it produces more ATP per oxygen consumed than fat[235]. There are multiple factors leading to mitochondrial dysfunction in HF, but elevated ROS production and oxidative damage[227–229], as well as defective mitochondrial biogenesis[236] are recurring themes, although whether these are causes or consequences of HF is less clear[237].

Mitochondrial dysfunction in HF could be targeted by preventing mitochondrial damage, increasing mitochondrial biogenesis or enhancing the ATP output of the remaining mitochondria[32,230,226,238,239]. As mitochondrial ROS production and oxidative damage have been found repeatedly in HF, the use of antioxidants to prevent this damage is an appealing strategy. Although this approach has worked in animal trials of HF, in translation to humans the results have generally been disappointing[230,239]. One way to enhance antioxidant effectiveness may be to target them to mitochondria[230,239], and supporting this possibility, MitoQ[193] and the mitochondria-targeted peptide Bendavia (SS31) have shown efficacy in animal models of HF[238,240,241]. More positively, the Q-SYMBIO trial showed that using CoQ_{10} as an antioxidant improved heart function[25], although larger trials are required. Upregulating mitochondrial biogenesis (for example, by activating PGC1α[242]) is a further potentially interesting approach[226]. Thus, therapies targeted at protecting mitochondria or increasing their biogenesis in HF are promising areas for future development[230].

Protozoal infections

Protozoal infections are responsible for a number of medically, socially and economically important diseases, including malaria (*Plasmodium falciparum*), African sleeping sickness (*Trypanosoma brucei*) and Chagas disease (*Trypanosoma cruzi*)[243,244], which are common in Africa and South America. Given the lack of vaccines, drug toxicity and the emergence of resistance, the development of new therapies for such parasitic diseases represents an area of urgent unmet need[95]. Protozoan mitochondria are an attractive drug target because their mitochondria not only are essential for survival but also are quite different from those of their mammalian hosts[95,244,245]. Therefore, although the application of mitochondria-based therapies in this setting is quite different from the indications discussed above, in that they do not target human mitochondria, given the substantial unmet need and promising therapeutic potential, strategies targeted to *Plasmodium* and trypanosome mitochondria are discussed below (FIG. 3). In addition, it should be noted that in contrast to the approaches discussed for other diseases, the potential therapeutic strategies outlined below aim to impair, rather than restore, mitochondrial function.

P. falciparum, the protozoan that underlies malaria, infects 200 million people worldwide and kills some 0.4 million per year, but it remains somewhat neglected by drug developers. *Plasmodium* undergoes dramatic changes in mitochondrial metabolism and function depending on the stage in its life cycle and its host[95,244]. Within the human red blood cell, the protozoan contains a single, large mitochondrion, which is essential for survival[95]. The *P. falciparum* mitochondrion contains a stripped-down respiratory chain comprising a non-proton-pumping NADH dehydrogenase (ND2) that oxidizes NADH in the cytosol, as well as conventional cytochrome bc_1 and cytochrome oxidase complexes that contain subunits that are encoded by mtDNA[244] (FIG. 3). The bloodstream form of *P. falciparum* relies entirely on

glycolysis for ATP production, but its mitochondrion nevertheless contains an active F_oF_1-ATP synthase that acts in reverse as a proton pump to help sustain a mitochondrial Δp that is essential for mitochondrial protein import and viability[95,244]. The major role of the respiratory chain is to pass electrons from NADH to O_2 to resupply NAD^+ in order to sustain glycolysis[244]. As *P. falciparum* lack pyrimidine salvage pathways, they rely on the mitochondrial

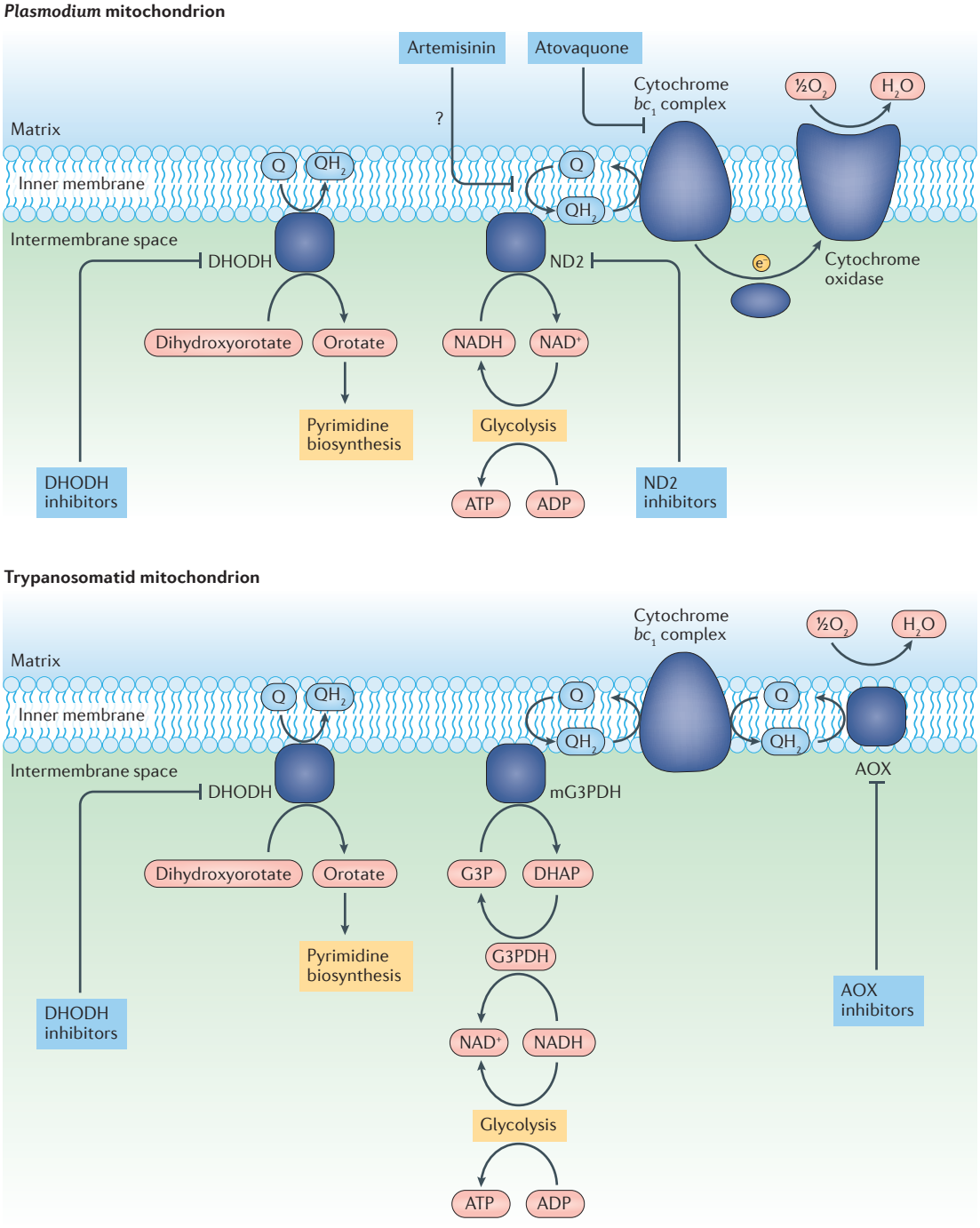

Figure 3 | **Mitochondria as a therapeutic target in protozoal infections.** The protozoan *Plasmodium falciparum* causes malaria whereas the trypanosomatids *Trypanosoma brucei* and *Trypanosoma cruzi* underlie sleeping sickness and Chagas disease, respectively. Several aspects of the metabolism of *Plasmodium* and trypanosomatid mitochondria are quite distinct from those in mammalian mitochondria, providing attractive druggable targets to treat protozoal infections. Therapeutic agents and strategies are shown in blue boxes. The ? indicates that this pathway is discussed as a possibility but is not proven. AOX, alternative oxidase; DHAP, dihydroxyacetone phosphate; DHODH, dihydroorotate dehydrogenase; e^-, electron; G3P, glyceraldehyde 3-phosphate; mG3PDH, mitochondrial glycerol 3-phosphate dehydrogenase; ND2, NADH dehydrogenase; Q, ubiquinone; QH_2, ubiquinol.

enzyme dihydroorotate dehydrogenase (DHODH) for pyrimidine biosynthesis[95,244,245]. DHODH is thus itself a potential drug target. Furthermore, as DHODH activity reduces ubiquinone (Q) to ubiquinol (QH_2), an active respiratory chain is also essential for pyrimidine biosynthesis by recycling QH_2 to Q[95,244,245].

The distinct and essential mitochondrial metabolism of *P. falciparum* immediately suggests that it should be a good drug target (FIG. 3) [95]. This suggestion is illustrated by the malaria drug atovaquone, which inhibits the *P. falciparum* cytochrome bc_1 complex more effectively than the mammalian complex[244]. However, rapid resistance to atovaquone occurs because its binding site on the cytochrome bc_1 complex is encoded by a gene on mtDNA that is more susceptible to oxidative damage and mutation, thereby facilitating the evolution of resistance[95,246]. This finding has led to the search for other *Plasmodium*-selective cytochrome bc_1 complex inhibitors and for ND2 inhibitors, with the latter less likely to generate resistance owing to the nuclear location of its gene[95,244,246]. The requirement for pyrimidine biosynthesis in *Plasmodium* has also led to the development of DHODH inhibitors[245,246]. One further interesting point to consider is that although the mode of action of the anti-*Plasmodium* drug artemisinin is unclear, it may act by disrupting mitochondrial respiration[247].

The trypanosomatid protozoa that underlie African (sleeping sickness) and American (Chagas disease) trypanosomiasis are widespread in Africa and South America, but as with malaria these devastating diseases are fairly neglected by drug developers. The mitochondria of trypanosomatids are an attractive drug target because they have different modes of metabolism, depending on host and stage of the life cycle, and are distinct from human mitochondria[243,248,249] (FIG. 3). The *T. brucei* trypomastigote stage in the bloodstream of infected humans, which relies on glycolysis for ATP production, contains a single mitochondrion that has an unconventional respiratory chain that is essential for regenerating NAD^+ from NADH to sustain glycolysis[249]. NAD^+ is regenerated from NADH by reduction of dihydroxyacetone phosphate to glycerol 3-phosphate by cytosolic glycerol 3-phosphate dehydrogenase[249]. The glycerol 3-phosphate is then reoxidized by mitochondrial glycerol 3-phosphate dehydrogenase (mG3PDH), thereby reducing mitochondrial Q to QH_2 which in turn is reoxidized by oxygen, catalysed by the AOX in the mitochondrial respiratory chain[243,250]. Trypanosomatid mitochondria also contain an active F_oF_1-ATP synthase that acts in reverse as a proton pump to maintain the Δp that is essential to maintain mitochondrial protein import and biogenesis[251]. The lack of AOX in humans makes it an appealing drug target[243,250] (FIG. 3); for example, the AOX inhibitor ascofuranone has been shown to be effective against *T. brucei* in mice in vivo[252].

There are likely to be many other potential targets in protozoan mitochondria distinct from those in human mitochondria; for example, some protozoans have unique metabolite transporters[108], and trypanosomatid mitochondria organize their mtDNA in concatenated chains, which makes them particularly sensitive to topoisomerase inhibitors[248].

Challenges

The development of mitochondrial therapies for common diseases faces considerable challenges. A key issue is the difficulty in assessing mitochondrial function and damage non-invasively in patients[253]. Currently, it can be difficult to know when to treat a patient with a mitochondrial therapy, or to determine whether the putative therapy acts on mitochondria or elsewhere[253]. There is an urgent need for biomarkers that are specific, sensitive over short periods of time and clinically meaningful[253].

To assess mitochondrial function, the most direct approach is to isolate mitochondria and assess their activity ex vivo — for example, as is done in muscle biopsies in the assessment of patients with mitochondrial disease. However, this is too invasive for repeated use; hence, there has been considerable effort devoted to assessing mitochondrial activity in blood leukocytes and platelets[254–256]. In these approaches, the full range of assessments of mitochondrial function or damage could be applied[254], but often now the approach is to subject the cells to bioenergetic profiling by respirometry to infer mitochondrial function[254,255,257]. This approach could in principle be applied directly to the assessment of mitochondrial function in these cell types, but more often the analysis of mitochondrial function in the blood is used as a surrogate marker for changes in mitochondrial activity in other, less accessible tissues, or as an indicator that a drug designed to affect mitochondria is effective in patients. These approaches are a current area of considerable interest, with the hope that measurements of mitochondrial function in the blood can be used to infer mitochondrial function and drug impact in other tissues.

Overall, mitochondrial function in the whole body can be assessed by changes in the blood or urine of the lactate: pyruvate ratio[258], and occasionally of changes of other metabolites, or by measuring markers of oxidative damage such as F_2-isoprostanes[259]. This assessment can be extended to link particular metabolic signatures in plasma and urine, which shows promise in some situations[260]. We may also be able to assess mitochondrial stress responses, such as changes in one-carbon metabolism that affect the release of fibroblast growth factor 21 (FGF21) or growth differentiation factor 15 (GDF15) into the circulation[6]. Other possibilities are the measurement of the release of mtDNA or mitochondrial-derived exosomes and microvesicles into the circulation[261]. However, a generic problem with these approaches is the difficulty of inferring the site of the tissue damage that led to release of the damage markers into the circulation. The ability to assess mitochondrial function in vivo has been approached in animals by targeting molecules to mitochondria to generate exomarkers[262,263], but as this requires the isolation of the tissue, its application to patients is currently limited to biopsy sample material[264].

Imaging technologies can be used to infer mitochondrial function within the tissues of interest in vivo. ^{31}P-Magnetic resonance spectroscopy (MRS) enables detection of ATP and creatine phosphate levels, which can be used to assess mitochondrial dysfunction in muscles and the brain[265,266]. Related to this is the endogenous assessment of mitochondrial oxygen consumption, which can be done in vivo with near-infrared spectroscopy

measurements[267]. Alternatively, mitochondrial function can be assessed by administering compounds to the patient and visualizing their distribution and metabolism. For example, positron emission tomography (PET) can be used to follow changes in mitochondrial membrane potential ($\Delta\psi$) in vivo by injecting a triphenylphosphonium (TPP) cation tagged with a PET-visible atom[268]. Alternatively, the transformations of ^{13}C-labelled metabolites can be assessed in vivo using MRS[266], and the sensitivity can be greatly enhanced by hyperpolarization of the ^{13}C-labelled metabolites before infusion[269]. The development of these and related approaches to assess mitochondrial function in vivo is central to the development of mitochondrial pharmacology.

Another major challenge in targeting drugs to mitochondria is how to achieve tissue selectivity so that the drug is delivered only to mitochondria in the tissue or cell type of interest, minimizing off-target effects. This can be addressed by the tissue-selective activation of a drug, as was done for DNP[176]. A related goal is to activate drugs only within mitochondria or to confine them there in order to minimize side effects[42]. These concerns are particularly acute when the intention is to kill cells such as protozoal parasites. There are a number of chemical biology approaches that suggest pathways towards these goals, such as selective activation of pro-drugs by enzymes, coadministration of multiple mitochondria-targeted compounds that react together within the organelle[270] or combination with other factors such as light or radiotherapy[271].

Outlook

Mitochondrial dysfunction can contribute to the pathology of many 'common' disorders, and general strategies by which small-molecule therapies targeting mitochondria may be used to treat these secondary mitochondrial diseases are emerging. This raises the prospect of treating common pathologies of considerable social, medical and economic importance with novel mitochondria-targeted therapies.

An appealing opportunity is raised by the repeated finding that mitochondria contribute to pathology by elevated ROS production, oxidative damage, carbon stress, disruption to calcium homeostasis, induction of the MPTP, the accumulation of protein aggregates and elevated inflammation. This suggests that a similar pattern of mitochondrial damage underlies disparate pathologies, enabling 'mitochondrial' drugs to be applied to many pathologies. A particularly intriguing corollary is that these same hallmarks of mitochondrial dysfunction are also found in organismic ageing and cell senescence[272]. This raises the possibility that mitochondrial drugs may increase overall health span. For example, the US National Institute on Aging (NIA) Intervention Testing Program (ITP)[273] showed that metformin in conjunction with rapamycin increased healthy lifespan[274,275], and now other mitochondrial drugs such as MitoQ are being assessed in the NIA ITP (see Related links). It will be interesting to see how these interventions affect 'normal' ageing and health span, raising the possibility of extending any promising findings with mitochondrial therapies in animals to prophylactic treatments to enhance the well-being of our ageing populations[276].

Of course, we have considered only a small number of the many possible diseases and indications for which mitochondrial therapies may be useful. For example, a major issue with many drugs is mitochondrial toxicity, which leads to the hepatotoxicity of acetaminophen[277], the heart damage caused by some cancer drugs[278] and the damage associated with antiretroviral therapies[74]. Coadministration of compounds designed to protect mitochondria may enable the wider use of drugs that are currently too toxic for routine use[279]. In addition to the many common disorders discussed throughout this Review that have a fairly clear 'physical' aetiology, a further intriguing possibility is that mitochondrial dysfunction may also contribute to psychological and psychiatric disorders such as anxiety and depression[280,281]. How mitochondrial dysfunction can affect mental processes is obscure at present but raises the prospect that intervening at the mitochondrial level may affect psychological and psychiatric disorders[280,281]. Time will tell whether focusing on mitochondria will provide new approaches to treat these and other common pathologies beyond the scope of this Review.

In conclusion, we have shown how we can think anew about therapies for common pathologies. Our view is that focusing on mitochondria and developing the field of mitochondrial pharmacology offers hope for new therapies in many of the most important pathologies facing humanity.

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Competing interests
M.P.M. has a financial interest in Antipodean Pharmaceuticals, a company that is developing mitochondria-targeted therapies. M.P.M. and R.C.H. also hold patents in the area of mitochondrial therapies. In addition, M.P.M. consults for Novintum Biotechnology, Cayman Chemicals and Takeda Pharmaceuticals, and R.C.H. consults for Cayman Chemicals.

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