Molecular mechanisms and consequences of mitochondrial permeability transition

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Abstract | Mitochondrial permeability transition (mPT) is a phenomenon that abruptly causes the flux of low molecular weight solutes (molecular weight up to 1,500) across the generally impermeable inner mitochondrial membrane. The mPT is mediated by the so-called mitochondrial permeability transition pore (mPTP), a supramolecular entity assembled at the interface of the inner and outer mitochondrial membranes. In contrast to mitochondrial outer membrane permeabilization, which mostly activates apoptosis, mPT can trigger different cellular responses, from the physiological regulation of mitophagy to the activation of apoptosis or necrosis. Although there are several molecular candidates for the mPTP, its molecular nature remains contentious. This lack of molecular data was a significant setback that prevented mechanistic insight into the mPTP, pharmacological targeting and the generation of informative animal models. In recent years, experimental evidence has highlighted mitochondrial F_1F_o ATP synthase as a participant in mPTP formation, although a molecular model for its transition to the mPTP is still lacking. Recently, the resolution of the F_1F_o ATP synthase structure by cryogenic electron microscopy led to a model for mPTP gating. The elusive molecular nature of the mPTP is now being clarified, marking a turning point for understanding mitochondrial biology and its pathophysiological ramifications. This Review provides an up-to-date reference for the understanding of the mammalian mPTP and its cellular functions. We review current insights into the molecular mechanisms of mPT and validated observations — from studies in vivo or in artificial membranes — on mPTP activity and functions. We end with a discussion of the contribution of the mPTP to human disease. Throughout the Review, we highlight the multiple unanswered questions and, when applicable, we also provide alternative interpretations of the recent discoveries.

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https://doi.org/10.1038/s41580-021-00433-y

Mitochondria are known to participate in a wide variety of cellular processes. Because they are the site of respiration, mitochondria are central regulators of cellular metabolism and participate in cell fate decisions. Mitochondria can activate the regulated cell death (RCD) pathways, especially those parts with apoptotic or necrotic features[1], and they are determinants of differentiation commitment or the stem cell pluripotent state[2]. Mitochondria also act as hubs for cellular signalling by actively mediating the flux of second messengers (for example, Ca^{2+} and cAMP)[3,4], serving as targets of signalling pathways, and producing small messengers, including reactive oxygen species (ROS)[5]. Notably, the inner mitochondrial membrane (IMM) is an extremely tight barrier. Therefore, all signalling molecules and mediators generated inside the mitochondria require a dedicated transporter to pass through the IMM. By contrast, the outer mitochondrial membrane (OMM), which envelopes the IMM, is more permeable to ions and small solutes via the activity of the voltage-dependent anion channel (VDAC), and specializes in selective protein diffusion to compartmentalize biochemical functions and signalling events[6–8].

Upon accumulation of Ca^{2+} in the mitochondrial matrix, a transition in the properties of the IMM is triggered, and it becomes permeable and poorly selective, allowing a variety of ions and solutes to be redistributed. This phenomenon is known as the mitochondrial permeability transition (mPT). This was originally believed to be an artefactual event, resulting from the degradation of the IMM, but we now know that mPT is mediated by a distinct entity with pore properties, the mitochondrial permeability transition pore (mPTP).

Opening of the mPTP can be reversed, which is regulated by the equilibrium between positive and negative modulators. However, the concentrations of

Voltage-dependent anion channel
(VDAC). A pore-forming protein of the outer mitochondrial membrane which allows the exchange of metabolites and ions.

Cyclosporine A
(CsA). A cyclic polypeptide able to bind and inhibit cyclophilins, including the mitochondrial permeability transition pore regulator cyclophilin D (CypD).

Adenine nucleotide translocator
(ANT). An integral membrane protein that exchanges ATP for ADP across the inner mitochondrial membrane.

Mitochondrial inorganic phosphate carrier
(PiC). An integral carrier solute that delivers phosphate across the inner mitochondrial membrane.

c-ring
A portion of the ATP synthase rotor (composed of an octamer of subunit c) responsible for the coupling of proton transport across the inner mitochondrial membrane to a rotative movement required for catalysis.

Cristae
Foldings of the inner membrane that create specialized compartments required to optimize mitochondrial functions.

these modulators vary according to different cellular conditions, setting the threshold at which mPT events become irreversible (TABLE 1). The mPT is important in the function of pathways that converge on and emerge from mitochondria, and has been implicated in many cellular events. Additionally, as a result of the discovery of its prototypical inhibitor, cyclosporine A (CsA)[9–13], we now know that mPT is involved in both the regulation of physiological cellular processes and the emergence of pathological conditions[14].

Here, we review knowledge of the mammalian mPTP, focusing on the current model for its molecular composition, its involvement in cellular pathophysiology and the available pharmacological toolbox for modulating its activity. Additionally, we discuss major flaws in the current model and uncertainties related to this intriguing mitochondrial phenomenon.

mPTP activation and modulation

The properties of mPT support the existence of one or a group of proteins responsible for the formation of the mPTP, although its molecular nature is still not fully understood. Many molecular components have been proposed, including the adenine nucleotide translocator (ANT), VDACs and mitochondrial inorganic phosphate carrier (PiC), although recent evidence has shown that models based solely on these components are improbable (BOX 1).

Molecular nature of the mPTP. In the past decade, attention has been focused on mitochondrial F_1/F_o ATP synthase (hereafter referred to as 'ATP synthase'), the enzymatic complex responsible for the synthesis of ATP from ADP and inorganic phosphate (P_i) in the IMM. ATP synthase is a multiprotein complex that consists of two well-defined regions: the soluble portion F_1, located in the mitochondrial matrix, including a catalytic part (the α–β trimer) and a regulative part (oligomycin sensitivity-conferring protein (OSCP)); the F_o portion straddles the IMM and includes the c-ring domain and the e, f and g subunits[15] (FIG. 1a).

The involvement of ATP synthase in mPTP formation is mostly based on three lines of evidence. First, isolated ATP synthase (or its c-ring domain), reconstituted in artificial bilayers, generates Ca^{2+}-inducible high-conductance currents[16–19]. Second, genetic manipulation of ATP synthase subunits in living cells markedly affects mPT[16,20–23]. Third, the mutagenesis of some ATP synthase subunits affects the dependence of the mPTP on some of its regulators[16,21,24–27].

From the data on mPT obtained thus far, the most accurate model foresees that the mPTP can open with a different configuration, each conferring a discrete set of conductances on the IMM, a phenomenon generally referred to as the 'multiconductance property' of the mPTP[28,29]. It is plausible to hypothesize that mPT is induced by two different types of pores (reasonably of different size), through which two types of currents pass: one at low conductance, with an approximate amplitude of 0.3–0.7 nS that allows the redistribution of ions (that is, protons, Ca^{2+} and K^+) and small metabolites (for example, glutathione), which is involved mainly in mitochondrial changes under physiological conditions; and a second, at high conductance, with an amplitude of approximately 1.5 nS that, by permitting the passage of larger solutes (for example, sucrose)[22,30,31], has a greater impact on mitochondrial structure and function, ultimately leading to RCD. Both mPTP opening events are reversible[9,29,32].

While the high-conductance state of the mPTP is induced by extremely stressful situations that overwhelm the mitochondria, a low-conductance state occurs spontaneously during the normal physiological activity of mitochondria (for example, in excitable cells it is triggered by physiological Ca^{2+} accumulation inside the mitochondria). This spontaneous phenomenon involves an mPTP switch between an on state and an off state, called 'flickering'[33–35]. Flickering events are possible because as quickly as the low-conductance permeability is triggered, the mPTP can be swiftly turned off due to a redistribution of ions between mitochondria and the cytoplasm. Indeed, the low-conductance pores generated across the IMM allow extrusion into the cytosol of Ca^{2+}, the permissive activator of the mPTP (with both the low-conductance pore and the high-conductance pore)[36], and the entry of H^+ ions from the cytosol into the mitochondria, which, by lowering the mitochondrial matrix pH, favours pore closure.

ANT is now considered the most likely candidate for the generation of the low-conductance pore[31,37,38], while the full-conductance currents are instead attributed to a pore originating within ATP synthase (as discussed above). Although it is reasonable that these two mPTP types of pores are closely related, because ATP synthase and ANT interact in the so-called ATP synthasome, whether this interaction is involved in the mechanism of pore formation or in the determination of the conductance state is still unknown (FIG. 1b).

Mechanism and controversies of Ca^{2+}-induced pore formation. The mechanism of pore formation within ATP synthase remains the most debated point in the research on mPT. It was recently demonstrated that Ca^{2+} binds to a site located in the soluble, F_1 portion (close to the Mg^{2+}-binding site between α- and β-subunits) of ATP synthase to trigger mPTP opening[25,36,39,40], while no binding site has been reported for the c-ring domain, which is envisioned in one of the existing models to function as the pore-establishing unit of the mPTP (see later). In contrast to Ca^{2+}, other divalent cations, especially Mg^{2+}, Sr^{2+}, Mn^{2+} and Ba^{2+}, are strong mPT inhibitors. While inhibition by Sr^{2+}, Mn^{2+} and Ba^{2+} has been proposed to occur by obstructing the Ca^{2+} influx inside mitochondria, Mg^{2+} works via competitive inhibition at the same site as Ca^{2+} (REFS[9–11]).

ATP synthase is organized mostly in rows of dimers that shape the IMM in cristae. Evidence indicates that mPTP formation starts at the sites populated by ATP synthase dimers but requires their disassembly for successful opening[21]. Two working models of pore formation are currently proposed: one based on the 'dimer hypothesis' and the other based on the 'c-ring hypothesis', which is also called the 'death finger model'. The first hypothesis states that Ca^{2+}-induced reconfiguration of

Table 1 | **Activators and inhibitors of the mammalian mPTP**

Molecule	mPTP regulation	Proposed target	Efficacy in mouse model of human disease	Refs
Endogenous regulators				
ADP	Negative	ANT; ATP synthase	NA	9–11,17,31,37
AMP	Negative	Unknown (possibly the same for ADP)	NA	9–11,17,31
ATP	Negative	Unknown (possibly the same for ADP)	NA	9–11,17,31
Creatine[a]	Negative	mCK	NA	240
H^+	Negative	His112 on OSCP	NA	26
Mg^{2+}	Negative	It competes with Ca^{2+} at its regulatory binding site	NA	9,25
Ca^{2+}	Positive	Two different binding sites for the regulation of mPT were predicted: one is demonstrated on β-subunit of ATP synthase; the second remains unknown	NA	9,25
Fatty acids	Positive	Unknown	NA	61–63
P_i	Positive	Unknown	NA	56–58, 241,242
ROS	Positive	Two distinct targets were proposed: one is dependent on pyrimidine oxidation and is unknown; another is dependent on thiol oxidation, and both GSH and CypD have been proposed	NA	51–53,55
Pharmacological regulators				
1,3,8-Triazaspiro[4.5] decane-derivative, compound 10	Negative	ATP synthase subunit c	Ex vivo model of ischaemia–reperfusion injury (Langhendorff heart perfusion)	174
Isoxazole-derivative, compound 63 (PubChem CID 75204518)	Negative	Unknown, probably not CypD	Ex vivo model of ischaemia–reperfusion injury (Langhendorff heart perfusion)	173
Bz-423 (PubChem CID 644335)	Positive	ATP synthase subunit OSCP	Mouse model of hereditary spastic paraplegia type 7 (*Spg7*-knockout mice)	17,243
Urea-base cyclophilin inhibitor, compound 19 (PubChem CID 72771088)	Negative	CypD	NA	244
Small molecule cyclophilin inhibitor, compound 31 (PubChem CID 90306602)	Negative	CypD	In vivo model of hepatic ischaemia–reperfusion injury	171
4-Aminobenzenesulfonamide derivative, C-9	Negative	CypD	NA	206
CsA (PubChem CID 5284373)[b]	Negative	Tryptophan residue (Trp121) of CypD	In vivo model of cardiac ischaemia–reperfusion injury In vivo model of acetaminophen-induced liver injury In vivo model of brain ischaemia–reperfusion injury (middle cerebral artery occlusion) In vivo model of oxalate-induced acute kidney injury Model of myopathy related to collagen VI deficiency	152,177,235, 245–247
Debio 025 (PubChem CID 11513676)	Negative	CypD	In vivo model of cardiac ischaemia–reperfusion injury Model of myopathy related to collagen VI and collagen VII deficiency Model for Duchenne muscular dystrophy (mdx mouse)	170,248–251
Dexpramipexole (PubChem CID 59868)	Negative	ATP synthase β-subunit and subunit OSCP	NA	252

Table 1 (cont.) | **Activators and inhibitors of the mammalian mPTP**

Molecule	mPTP regulation	Proposed target	Efficacy in mouse model of human disease	Refs
Pharmacological regulators (cont.)				
Gamitrinibs	Positive	HSP90	Orthotopic model of bone metastatic prostate cancer	253
GNX-4728	Negative	Unknown, possibly ANT	Transgenic model of amyotrophic lateral sclerosis (expressing human $SOD1^{G37R}$)	205
GNX-4975	Negative	Unknown, possibly ANT	NA	254
JW47	Negative	CypD	In vivo model of experimental multiple sclerosis	255
ML-404	Negative	Unknown, probably not CypD	NA	256
NIM-811 (PubChem CID 6473876)	Negative	CypD	In vivo model of skeletal muscle ischaemia–reperfusion injury In vivo bone fracture model In vivo model of kidney ischaemia–reperfusion injury In vivo model of brain ischaemia–reperfusion injury (transient focal cerebral ischaemia) In vivo model of experimental multiple sclerosis	169,257–260
Sanglifehrin A (PubChem CID 5388925)[b]	Negative	CypD	In vivo model of cardiac ischaemia–reperfusion injury	261
SB216763 (PubChem CID 176158)	Negative	GSK3β	Ex vivo model of ischaemia–reperfusion injury (Langhendorff heart perfusion)	262
TR002	Negative	Unknown, probably not CypD	NA	173
VDAC1-based peptides	Positive	HKII	Xenograft model of glioblastoma	263

ANT, adenine nucleotide transporter; CsA, cyclosporine A; CypD, cyclophilin D; GSH, reduced glutathione; GSK3β, glycogen synthase kinase 3β; HKII, hexokinase II; mCK, mitochondrial creatine kinase; mPT, mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; NA, not available; OSCP, oligomycin sensitivity-conferring protein; P_i, inorganic phosphate; ROS, reactive oxygen species; VDAC1, voltage-dependent anion-selective channel protein 1. [a]Inhibition of mPT by creatine was reported in transgenic mice overexpressing mCK, but not in wild-type mice. [b]Displays significant affinity for other cyclophilins (for example, cyclophilin A).

the dimer causes the formation of a pore at the interface of two ATP synthase monomers (FIG. 1c). The second hypothesis proposes that the conformational change induced by Ca^{2+} allows the c-ring to generate the pore (FIG. 1c).

Different studies provided criticisms of both these models, raising doubts about their validity. Genetic deletion of ATP synthase subunits that impede the formation of the c-ring or the peripheral stalk (the portion responsible also for the formation of dimers; FIG. 1a) did not block the formation of Ca^{2+}-sensitive and CsA-sensitive non-selective pores in the IMM[41,42]. Lately, it was demonstrated that ion currents observed in mitochondria in cells in which subunit c (the building block of the c-ring) has been knocked out are characterized by significantly lower amplitude than the high-conductance state of the mPTP[31]. These low-amplitude currents are now proposed to be formed by ANT as they are sensitive to the ANT antagonists and since ANT was originally reported to generate low-conductance currents in artificial membranes. In agreement, genetic deletion of ANT isoforms, mitochondrial matrix protein cyclophilin D (CypD; an essential component of the mPTP)[37] and ATP synthase subunit g[38] has now strengthened the model that ANT and ATP synthase might be independent pores responsible for low and high mPTP conductance, respectively.

The resolution of the bovine dimeric ATP synthase and mathematical modelling of its behaviour indicated that the c-ring lumen is enriched in hydrophobic residues and its core is occupied by a lipid plug (FIG. 1a); this dense structure is held in place by a fragment of subunit e, and would act as a plug filling the cavity of the c-ring, thus making it difficult to envision the role of the c-ring in the establishment of the pore[15,43]. The death finger mode of pore opening at the c-ring originally proposed that significant rearrangements of the ATP synthase structure might lead to the expulsion of this dense c-ring core and rearrangement of the residues exposed in the c-ring lumen, leading to the formation of a pore permeable to solutes[44]. In support of this model, the resolution of the mammalian ATP synthase structure by cryogenic electron microscopy revealed, in the presence of Ca^{2+}, the formation of aberrant ATP synthase displaying a shift of subunit e and an exposed c-ring, apparently deprived of the lipid plug. Cryogenic electron microscopy data also indicate that the c-ring appears enlarged, although the mechanism by which the hydrophobic lumen becomes hydrophilic remains an open question. As subunit e and subunit g are required for the

Cyclophilin D
(CypD). A peptidyl-prolyl *cis–trans* isomerase located in the mitochondrial matrix. By binding to mitochondrial ATP synthase, it functions as a positive regulator of mitochondrial permeability transition pore (mPTP) opening and the target of the mPTP inhibitor cyclosporine A (CsA).

Mitoplasts
Mitochondria artificially deprived of the outer mitochondrial membrane.

dimerization of the ATP synthase and are proposed to be the site of pore formation, the reported rearrangement of subunit e leaves open the possibility of the formation of a pore at the interface between dimers[39].

The current emerging picture thus proposes that Ca^{2+} binding to ATP synthase causes a modification of the conformation of the complex, which allows the formation of the high-conductance polar pore within the F_o portion[39], alongside ANT pores that are responsible for the low-conductance state (FIG. 1b). Although controversies are still to be addressed, we now have a solid model of the mPTP, which can now serve to design novel and more specific experiments to obtain more accurate, biologically relevant mechanistic insights into permeabilization of the IMM.

Molecular modulation of mPTP function. While it is well established that mitochondrial Ca^{2+} is the trigger for mPTP opening, under physiological conditions, mitochondria can accumulate large amounts of Ca^{2+} without experiencing mPT. Indeed, different endogenous modulators of mPT can modify the threshold for Ca^{2+} concentration required to trigger that event.

Adenine nucleotides are probably the most potent endogenous inhibitors of mPT. ADP, ATP and AMP (listed from strongest to weakest) all affect mPT by both decreasing the rate of permeability propagation and increasing the Ca^{2+} concentration required to trigger mPT[10,45]. The mechanism responsible for this inhibition is still not fully understood, although it is believed

Box 1 | **History of the mPTP: successes and failures in mPTP component discovery**

The molecular nature of the mitochondrial permeability transition pore (mPTP) has been investigated extensively over the past 40 years (see the figure), and different models have been proposed. All of them inevitably have fallen short when challenged by genetic models.

The earliest search for the mPTP structure was based on adenine nucleotide transporter (ANT). Two studies in the early 1990s demonstrated that ANT could be isolated in complexes with the outer mitochondrial membrane (OMM) channels voltage-dependent anion channel (VDAC) and translocator protein (TSPO)[277] and that different benzodiazepines targeting TSPO were capable of inducing or inhibiting mitochondrial permeability transition (mPT)[280]. Subsequently, ANT was found in complex with VDAC, hexokinase II (HKII), mitochondrial creatine kinase (mCK) and cyclophilin D (CypD), and when reconstituted in liposomes, it facilitated permeability to solutes or currents with properties resembling those of the mPT[281,282]. An initial model was then established, depicting the mPTP as a complex formed at contact sites between the inner mitochondrial membrane (IMM) and the OMM, with VDAC and ANT forming channels in the two membranes and TSPO, HKII, mCK and CypD involved in stabilizing and regulating the complex.

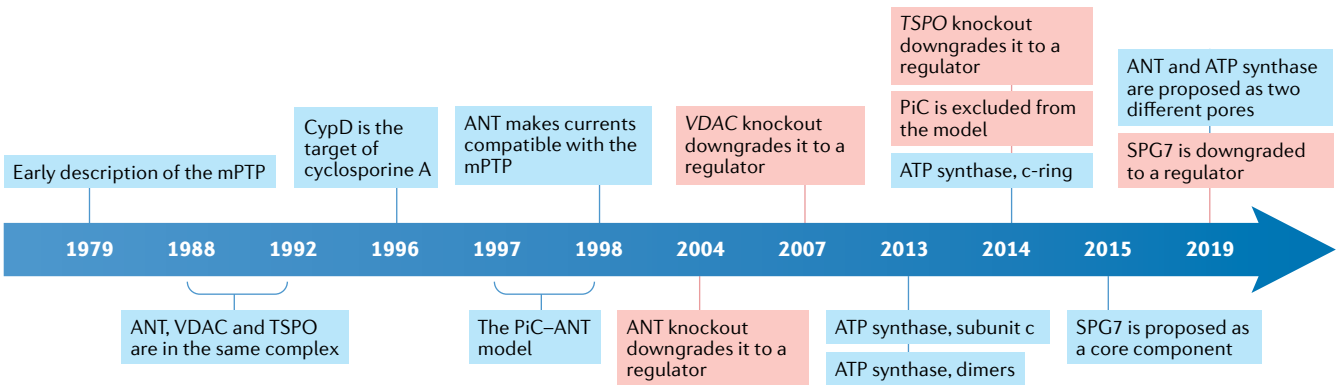
In 2004, the double-knockout mouse model for ANT isoforms 1 and 2 revealed the persistence of Ca^{2+}-inducible mPT (although with an increased threshold for Ca^{2+} induction and insensitivity to ADP or atractyloside), downgrading ANT from a pore-forming candidate to a regulator of the complex[46]. Genetic deletion of all VDAC isoforms and conditional knockout of *TSPO* clearly showed that the properties of the mPTP were unchanged by VDAC[276] or TSPO[283] deprivation, discrediting a model of the mPTP that had been used for a few decades. The exclusion of VDAC and TSPO from the mPTP complex started a new controversy. Indeed, it was demonstrated that certain compounds targeted mPTP regulators on the OMM (possibly VDAC and TSPO), as demonstrated by the loss of their effect on mitoplasts. This problem has not been solved yet.

Another model that received significant scrutiny was based on mitochondrial inorganic phosphate carrier (PiC), which was found to bind CypD and ANT and was able to generate Ca^{2+}-induced currents[284], leading to a model in which PiC, modulated by CypD, formed the pore in response to Ca^{2+} through interactions with ANT[285]. This model immediately raised some concerns, as the currents generated by the reconstituted PiC displayed a smaller amplitude than that reported for the mPTP. Furthermore, cardiac-specific deletion or overexpression of PiC did not affect mPT. It is now proposed that PiC might modulate mPTP opening by regulating the matrix levels of inorganic phosphate[286].

A few years ago, small interfering RNA-based screening revealed spastic paraplegia type 7 protein (SPG7) as a novel component of the mPTP. Its genetic inactivation increased resistance to Ca^{+}-induced mPT in permeabilized cells, and SPG7 was found in a complex with CypD and VDAC[268]. Independent investigators later failed to replicate the observation in isolated mitochondria[287], and while a regulatory effect of SPG7 on the mPTP was confirmed in intact cells[243], its mechanism appears to be related to the control of mitochondrial Ca^{2+} uptake[269].

In the past decade, multiple lines of evidence have resulted in the proposal that the mPTP forms via a rearrangement of ATP synthase. This model has been confirmed by independent laboratories, although there is still debate on the exact mechanism of pore formation[31] (see the main text). Recently, the generation of a mouse triple knockout for all the different ANT isoforms indicated that the transporter might represent an independent pore responsible for the low-conductance mPTP[37]. Nevertheless, considering that ANT and ATP synthase exist in the same complex (ATP synthasome, where PiC is also found), further investigations might provide novel and significant insights into the mechanism of mPTP formation.

A definitive resolution of the mPTP structure will allow the definition of those phenomena strictly related to the mPTP rather than other functions of mPTP components and regulators. The knockout mouse exemplifies this for CypD, which is already proposed to have phenotypes independent of mPTP activity, which should always be considered carefully[288].



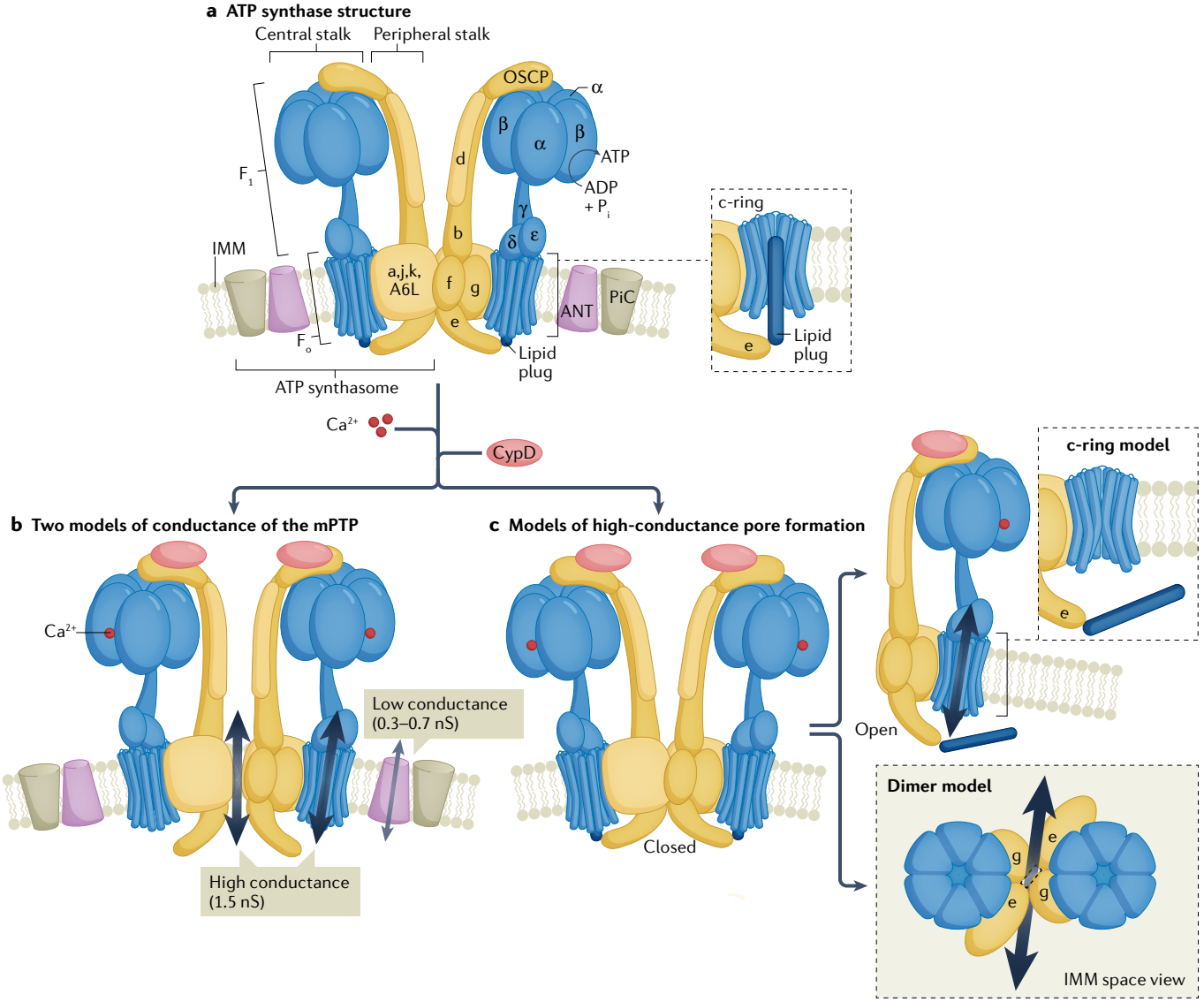


Fig. 1 | **ATP synthase structure and mechanism of mPTP opening.** **a** | Cartoon depicting the structure of ATP synthase. The central stalk contains the catalytic α3–β3 domain, the rotor (γ, δ and ε trimer) and the c-ring and is responsible for the rotatory conformational change that transduces the proton gradient across the inner mitochondrial membrane (IMM) for the catalysis of ATP synthesis. The peripheral stalk offers a reference system between the rotor and the catalytic portion of the central stalk and participates in dimer formation and in proton transport. The F_1 portion is composed mostly of a water-soluble subunit, while F_o is integral in the IMM. ATP synthase forms stable interaction with adenine nucleotide transporter (ANT) and inorganic phosphate carrier (PiC) in complexes named 'ATP synthasomes'. The inset depicts a sectioned c-ring and displays a high-density structure observed by cryogenic electron microscopy. This structure (possibly composed of lipids) is in contact with subunit e and impedes any passage of solutes across the c-ring, thereby serving as a 'plug' for the channel formed by the ring structure. **b** | Models of high-conductance and low-conductance pore formation by ATP synthase and ANT. ANT and ATP synthase are believed to participates in low-conductance and high-conductance mitochondrial permeability transition pore (mPTP) currents, respectively, as independent channels. **c** | Models of high-conductance pore formation from ATP synthase. The binding of Ca^{2+} and cyclophilin D (CypD) to ATP synthase causes the deformation of the monomers and destabilization of its dimeric structure. At this point two possible explanations for the pore formation are proposed. The c-ring model proposes the deformation of subunit e causing the release of the lipid plug (see inset in panel **a**) from the c-ring, which becomes the pore. The dimer hypothesis proposes that the pore is formed at the interface between subunit g and subunit e of two interacting monomers. OSCP, oligomycin sensitivity-conferring protein; P_i, inorganic phosphate.

Mitochondrial membrane potential
The electric potential generated across inner mitochondrial membrane by the proton pumping activity of respiratory complexes.

to be mediated by ANT[37,46] or the c-ring domain[16]. Acidification of the mitochondrial matrix also inhibits mPT, with the optimum pH for pore opening estimated to be 7.4 (REF.[9]) Inhibition of the mPTP by low pH is now recognized as being caused by the protonation of His112 on the regulatory OSCP subunit of the ATP synthase complex[26].

The mitochondrial membrane potential is another factor that modulates mPTP opening. Induction of mPT is possible in both de-energized and fully respiring mitochondria, although in the second case, mPT induction is significantly desensitized, which is due to the dependence of the mPTP on the membrane potential of the IMM. Physiological values of the mitochondrial

BCL-2 family
A group of evolutionarily conserved proteins that harbour a BCL-2 homology domain. Mostly known for their regulatory role in regulated cell death, mostly apoptosis.

Sirtuin
Member of a family of NAD-dependent protein deacetylases or (ADP-ribosyl) transferases able to respond to nutrient stress, potentiating mitochondrial biogenesis and activity.

ERK-mediated signalling
A signal transduction pathway regulating cellular processes that includes proliferation, differentiation, apoptosis and stress responses, having a major impact in tumour development.

membrane potential keep the pore closed, while a reduction in mitochondrial membrane potential favours the transition to spontaneous induction of the mPT[32,33,47]. The existence of a designated voltage sensor of the mPTP was proposed long ago[48,49] yet is still undetermined, although one study proposed that it might reside in the c-ring domain[16].

The best characterized positive modulator of the mPTP is the chaperone CypD[50]. This protein binds to the OSCP subunit, and it is proposed to favour a conformational rearrangement of ATP synthase, which ultimately results in mPTP opening[25]. Accordingly, CypD is thought to be an important component of the mPTP. The gold-standard inhibitor of the mPTP, CsA, by binding CypD induces its detachment from ATP synthase, disfavouring pore formation. By contrast, the benzodiazepine Bz-423, a novel proapoptotic drug for immunomodulation in the treatment of lupus erythematosus, binds to OSCP, mimicking CypD and favouring mPTP opening[17]. Other important endogenous positive regulators of the mPTP include ROS, P_i and fatty acids. Oxidative stress is probably the most thoroughly described inducer due to its pathophysiological implications, and several pro-oxidants have been reported to favour Ca^{2+}-induced mPTP opening[51,52]. The use of pyrimidine nucleotides and dithiols allowed the identification of two different sites for redox modulations (called the 'P site' for pyridine nucleotides and the 'S site' for dithiols), both of which have been proposed to be distant from the Ca^{2+}-triggering site[53]. Thiol-oxidizing agents have been shown to decrease the affinity of the mPTP for ADP, therefore repressing its inhibition potential and increasing the binding affinity for CypD[54]. Initial observations addressed the impact of thiol oxidants on glutathione, but more recent observations proposed Cys141 of OSCP as one of these sites[24,55]. The mechanisms responsible for both P_i synergism and fatty acid synergism with Ca^{2+} to induce mPTP opening remain largely unclear[55]. Stimulation with P_i can induce ROS production together with mPT, and P_i-induced mPT is prevented by administration of the antioxidant catalase. Additionally, oxidative stress potentiates mPT induced by P_i, suggesting that ROS, at least partially, are involved[56–58]. Multiple mechanisms have been proposed for fatty acids, involving the participation of ANT, VDAC, BAX (a member of the proapoptotic BCL-2 family), several kinases[59–63] and the regulation of voltage-sensing mechanisms of the mPTP; however, indisputable evidence is still missing.

Regulation of mPTP by proteins and signalling networks. Although the molecular composition of the pore is still under investigation, several protein regulators of mPT have been identified that can either directly bind to mPTP components or drive post-translational modifications of mPTP or its regulators (TABLE 2).

In particular, a number of interactors and post-translational modifications of the CypD chaperone have been described to affect mPT. CypD appears to be involved in a network of chaperones controlling mPT. CypD was demonstrated to interact with the mitochondrial chaperonins HSP60 and HSP90 as well as DNAJ homologue subfamily C member 15 (DNAJC15). Both HSP60 and HSP90 antagonize CypD to confer protection against mPTP opening and cell death[64,65]. By contrast, the interaction of DNAJC15 with CypD positively regulates the mPTP[66]. Interestingly, HSP90 negatively regulates OSCP stability, suggesting that it could also modulate the interaction between CypD and OSCP[67].

In vitro and in vivo, the sirtuin SIRT3 interacts with and deacetylates CypD at Lys166, increasing the threshold for Ca^{2+}-induced mPT (although in vivo the effect of SIRT3 on mPT appears to be more pronounced in aged mice than in young mice)[68,69]. In addition, SIRT3 mediates pH-dependent deacetylation of Lys70 on the OSCP subunit, impairing its interaction with CypD[70,71]. Control of CypD-dependent mPT also depends on its phosphorylation status. A pool of GSK3β, a ubiquitously expressed serine/threonine kinase, has been proposed to localize to the mitochondrial matrix and phosphorylate CypD in response to ERK-mediated signalling[72], favouring its interaction with OSCP[73] and sensitizing mPT-induced and Ca^{2+}-induced cell death (see the section entitled Cellular consequences of mPTP opening)[74–78]. By contrast, activation of the PI3K pathway resulted in activation of AKT2, which phosphorylated CypD at Ser31, potentially negatively affecting mPTP opening[79].

Other kinases have been shown to suppress mPTP opening, especially mitochondrial creatine kinase (mCK), hexokinase II (HKII), protein kinase Cε (PKCε) and protein kinase A (PKA)[80–84] (BOX 1). For all these kinases, the mechanism of action (including any phosphorylation sites in mPTP components or modulators) is unknown, but the interaction with ANT seems to be a driving point for some of them. Interestingly, all of these kinases can also control energy metabolism, suggesting that signal transduction pathways may have evolved to exert coordinated control of energy production and mitochondrial stress tolerance via mPT.

Additionally, multiple members of the BCL-2 family have been reported to regulate mPTP opening. The proapoptotic members of the family, BAX, BAK and BAD, are all positive regulators of mPT[85–87] (see the next section). Antiapoptotic BCL-X_L has been reported to interact with ATP synthase, favouring its enzymatic activity in ATP production[88], and has been proposed to disfavour the molecular transition from ATP synthase to the mPTP[89]. These findings raise the intriguing possibility that BCL-2 family members can have a direct impact on ATP synthase conformation and therefore mPTP formation, although further experiments are required to confirm this hypothesis.

All these data indicate the need to integrate the activity of the mPTP in the whole picture of cell physiology — via signalling networks — to maximize its impact in the adaptation of cell function to different contexts.

Mitochondrial changes induced by mPT

Upon opening of the mPTP, any molecule that exhibits a gradient across the IMM may passively diffuse into the intermembrane space and later, via the semipermeable OMM, into the cytoplasm. It follows that mPT has a significant impact not only on the physiology of mitochondria but also on the whole cell (see the next section).

Table 2 | **Proteins reported to modulate the mPTP**

Protein	mPTP regulation	Molecular mechanism	Refs
AKT	Negative	Mediates association of HKII with outer mitochondrial membrane	264
BCL-X_L	Negative	Unknown; it is proposed that it stabilizes the ATP synthase structure to impede mPTP formation	89
ERK	Negative	Phosphorylates and inhibits GSK3β	72,265
HIF1α	Negative	Upregulates HKII	145
HKII	Negative	Unknown; it is proposed to act on VDAC	82,263,266
HSP60	Negative	Mediated by the interaction with CypD	105
HSP90	Negative	Mediated by the interaction with CypD	106
mCK	Negative	Unknown	80,240
PKA	Negative	Phosphorylates VDAC, although is not clear if this phosphorylation directly affects mPT	84
PKCε	Negative	Apparently mediated by GSK3β	75,83,267
SGK1	Negative	Phosphorylates VDAC, sending it for proteasomal degradation	223,224
SIRT3	Negative	Deacetylates CypD and OSCP, blocking their interaction	13,68,70,71
SPG7	Positive	Interacts with CypD, VDAC and ANT; also regulates mitochondrial Ca^{2+} homeostasis	268,269
TRAP1	Negative	Mediated by the interaction with CypD	106
CypD	Positive	Reduces the Ca^{2+} affinity of the mPTP via an unknown mechanism. The binding with CsA detaches CypD from ATP synthase, and it is postulated that this can cause conformational rearrangement of ATP synthase to expose Ca^{2+}-binding sites	13,248,257,261,270–274
DNAJC15	Positive	Mediated by the interaction with CypD	66
GSK3β	Positive	Mediated by the phosphorylation of CypD	262
p53	Positive	Mediated by the interaction with CypD	13,235,275
VDAC	Positive	Unknown; it is hypothesized that it could stabilize the mPTP complex, although its presence is not mandatory for mPT	32,276,277
BAX	Positive	Mediated by the interaction with ANT	278
BAK	Positive	Mediated by the interaction with VDAC	279
BAD	Positive	Unknown, although evidence indicates the participation of PKA and PKC	87

AKT, RACα serine/threonine-protein kinase; ANT, adenine nucleotide transporter; BCL-X_L, BCL-2-like protein 1, isoform L; CsA, cyclosporine A; CypD, cyclophilin D; DNAJC15, DNAJ homologue subfamily C member 15; ERK, extracellular-signal-regulated kinase; GSK3β, glycogen synthase kinase 3β; HIF1α, hypoxia-inducible factor 1α; HKII, hexokinase II; HSP, heat shock protein; mCK, mitochondrial creatine kinase; mPT mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; OSCP, oligomycin sensitivity-conferring protein; PKA, protein kinase A; PKC, protein kinase C; SGK1, serum/glucocorticoid-regulated kinase 1; SPG7, spastic paraplegia type 7 protein; TRAP1, tumour necrosis factor type 1 receptor-associated protein; VDAC, voltage dependent anion channel.

Physiological mPTP flickering is a phenomenon that, by offering an alternative route for Ca^{2+} efflux from the mitochondrial matrix, can control the amount of Ca^{2+} inside the mitochondria and in turn Ca^{2+}-related metabolism (FIG. 2). Moreover, the low-conductance mPTP opening permits the extrusion of endogenous ROS[90] and other molecules produced inside the mitochondria into the cytoplasm, where they impact cell physiology, as described in the next section.

Conversely, persistent and widespread opening of the high-conductance mPTP has a profound impact on the activity of mitochondria, particularly on the respiratory chain, causing depletion of NADH[91,92] — the major source of electrons for respiratory chain activity — as well as inducing the disassembly of respiratory supercomplexes[93], which are aggregates of respiratory complexes that are thought to increase the efficiency of electron flow within the respiratory chain. The resulting impairment of electron flow, especially within complex I, leads to an increase in ROS production, which soon exceeds the physiological level associated with ROS signalling[94].

Accordingly, the prolonged opening of the mPTP causes a detrimental impairment of mitochondrial physiology and energy metabolism. When the mPTP is opened to high conductance, the redistribution of ions and small molecules causes an inverse redistribution of H_2O (FIG. 2). Water entry into the mitochondrial matrix increases the osmotic pressure, leading to partial distension of the mitochondrial cristae and enlargement of the matrix volume, leading to mitochondrial swelling. Since the IMM has a much larger area than the OMM, mitochondrial swelling requires a profound remodelling of the OMM, otherwise opposing IMM distension. The original report documenting this

Respiratory chain
A group of multimeric protein complexes in the inner mitochondrial membrane, carrying mitochondrial respiration.

mPTP-induced mitochondrial swelling proposed that this event is accompanied by mechanical rupture of the OMM. However, it is now established that the loss of OMM integrity is mediated by BAX and BAK, which oligomerize to form pores that are large enough to permit the extrusion of the IMM[85,95].

Mechanistically, the dissipation of mitochondrial membrane potential caused by proton influx into the matrix influences mPTP voltage sensing, favouring its open state. In addition, dilution of the enzymes and metabolites caused by swelling results in an inhibition of the tricarboxylic acid (TCA) cycle and respiratory complex function, which are unable to counteract proton leakage. In the absence of a mitochondrial membrane potential and with the dilution of its substrates, ATP synthase stops ATP synthesis, causing rapid blockade of ATP-dependent reactions. At this stage, mitochondria are unable to generate the conditions needed to close the mPTP, making it irreversible, which in most cases results in cell death (FIG. 2).

Cellular consequences of mPTP opening

As described already herein, mPTP opening has crucial implications for mitochondrial structure and function, which are proportional to the magnitude of IMM permeabilization. Mitochondria are central for regulating cellular functions, from the control of intracellular messengers (for example, Ca^{2+} and ROS) and modelling of metabolism to dramatic processes as engagement of RCD. Then, is not surprising that the mPTP connects to the same processes.

Impact on mitochondrial bioenergetics and ROS production. One major implication of mPTP opening is alteration of cellular metabolism owing to the redistribution of ions and metabolites involved in biochemical reactions and the impairment of mitochondrial activity, which usually sustains multiple biochemical pathways in the cell. Deletion of CypD limits transient mPTP flickering[33], causing an elevation of mitochondrial Ca^{2+} levels that is sufficient to boost the TCA cycle[96].

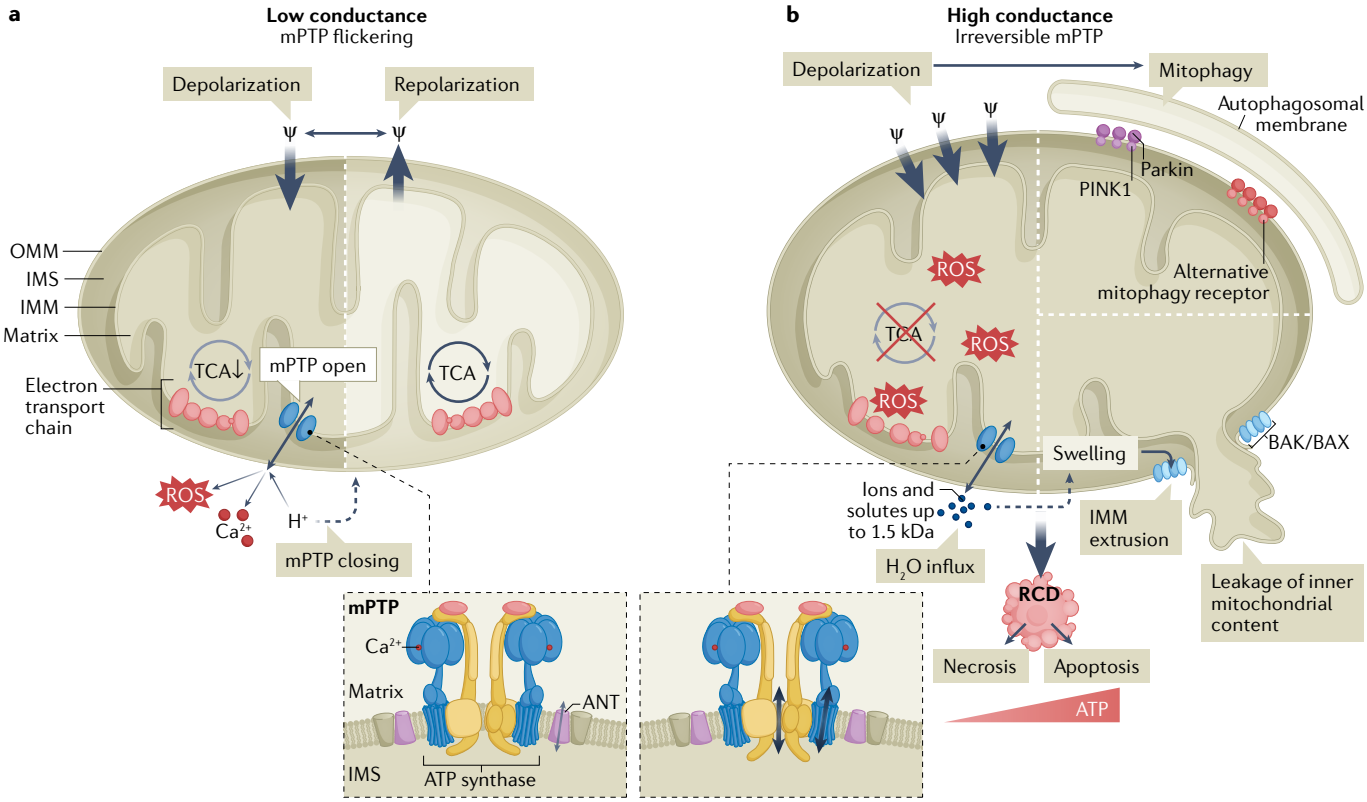


Fig. 2 | **Mitochondrial consequences of mPT. a** | The low-conductance current associated with mitochondrial permeability transition (mPT) allows redistribution of H^+ and Ca^{2+} across the inner mitochondrial membrane (IMM). Mitochondria experience brief depolarization (reduction of membrane potential ($\Delta\Psi m$)), which temporarily shuts down mitochondrial bioenergetics and contributes to generation of reactive oxygen species (ROS). However, the reduction of pH and intramitochondrial calcium concentration associated with mitochondrial permeability transition pore (mPTP) opening induces the closure of the mPTP and restoration of mitochondrial physiology. **b** | The high-conductance current of mPT causes complete and persistent mitochondrial depolarization, and redistribution of ions and solutes of up to 1.5 kDa across the mitochondrial membrane. In response, mitochondrial respiratory supercomplexes disassemble and respiratory complex I induces production of ROS (left). The large depolarization can induce the activation of mitophagy (driven by PINK1–parkin or alternative mechanisms) (top right), resulting in clearance of mitochondria affected by the mPTP (serving as a quality control mechanism against negative cellular consequences of mPT) (FIG. 4). If such mitochondria are not cleared, the widespread redistribution of ions eventually causes the influx of water and then the expansion of mitochondrial volume, with distension of the IMM and the disappearance of mitochondrial cristae. This activates the outer mitochondrial membrane (OMM) pore-forming proteins BAX and BAK, which then cause fenestration of the OMM, leading to its permeabilization, ultimately triggering regulated cell death (RCD) with necrotic or apoptotic features, depending on ATP availability. BAX/BAK oligomerization can also be associated with the extrusion of the IMM through the BAX/BAK pores. IMS, intermembrane space; TCA, tricarboxylic acid.

Consistent with this scenario, mitochondria isolated from mice carrying a deletion of the gene *Ppif* (which encodes CypD) display alterations in TCA cycle metabolites[96], glucose uptake[97], gluconeogenesis rates[98] and expression levels of the genes involved in branched-chain amino acid degradation, the TCA cycle and fatty acid oxidation[96,99]. It must be noted though that CypD targets other than the mPTP have also been proposed to regulate metabolism[96]. In addition, a high-fat diet in mice induces mitochondrial damage via mPT, causing insulin resistance[97] and liver accumulation of triglycerides[100].

As already mentioned, the mPT promotes ROS production. The sustained increase in ROS levels is detrimental for mitochondria and favours further ROS production via multiple mechanisms (a phenomenon called 'ROS-induced ROS production'). As an example, ROS can detach cytochrome *c* from IMM lipid cardiolipin, making it incapable of transporting electrons from complex III to complex IV. Electrons then accumulate across respiratory complexes I and III, further boosting their ROS production. ROS can diffuse to surrounding mitochondria that have not undergone mPT, lowering the threshold for mPTP opening and triggering a vicious cycle that can eventually lead to widespread cellular ROS production[101] and damaging oxidative stress (FIG. 2).

Induction of RCD. Widespread mPTP opening causes an energetic crisis in the cell that the reported increase in glycolytic rates cannot counteract. Furthermore, increased glycolytic activity leads to the accumulation of pyruvate and, therefore, lactate, causing acidification of the cytoplasm and mitochondrial matrix. The limited ATP availability impairs plasma membrane ATPases that actively pump Na^+, Ca^{2+} and H^+ outside the cytosol and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) that pumps Ca^{2+} within the endoplasmic reticulum lumen. Na^+ and Ca^{2+} gradients are then dissipated, and the slowdown of H^+ pumping contributes to cytoplasmic acidification. The dramatic loss of ion gradients ultimately leads to the collapse of the plasma membrane and cell death with necrotic features[1,102,103] (FIG. 2).

However, necrosis is not the only outcome of detrimental mPT. Indeed, the loss of OMM integrity induced by mitochondrial swelling (see the previous section) allows the release of proapoptotic factors into the cytosol: cytochrome *c*, mitochondrial apoptosis-inducing factor 1 (AIFM1), serine peptidase HTRA2, Diablo and endonuclease G. The release of these factors initiates the intrinsic pathway of apoptosis, ultimately leading to cell death[102,103]. Compelling evidence indicates that mPT can induce both routes of RCD[12,104–106], and the factor determining the choice between necrosis and apoptosis appears to be cellular ATP levels. Apoptosis is an energy-dependent process, and it was demonstrated that forcing the elevation of ATP production induces the switch from necrosis to apoptosis after mPT induction[107] (FIG. 2). Whether this choice is solely dependent on the energetic status of the cell or on the extent of mPT induction remains to be determined. In addition, while most data indicate that RCD results from high conductance-induced mitochondrial swelling, a formal demonstration that RCD is not induced by low conductance is still missing.

Induction of mitophagy. Opening of the mPTP can induce removal of mitochondria via mitophagy[108]. In different in vitro systems, depolarization of mitochondria leading to their incorporation into lysosomes has been observed upon induction of cell starvation, and this phenomenon is strongly inhibited by mPTP desensitizers such as CsA or its analogues[109,110]. Elevation of cytoplasmic Ca^{2+} levels and oxidative stress — both closely associated with mPT — are related to induction of the autophagic response[111,112] and are the most likely promoters of mPTP-related mitophagy.

Multiple molecular routes are described to deliver damaged mitochondria to lysosomes for degradation; the best characterized is the PINK1–parkin system[113]. This mechanism has been proposed to mediate mPTP-induced mitophagy after deletion of the mitochondrial fission protein DRP1 (REF.[114]) or laser-induced ROS production (FIG. 2). Interestingly, carbonyl cyanide *m*-chlorophenylhydrazone-induced mitochondrial depolarization — a potent mPTP stimulus — results in CsA-independent recruitment of parkin to mitochondria[115], suggesting that mPTP opening is not the only route for activation of mitophagy in stressed mitochondria.

Mitophagy also impacts mPT, as its genetic inactivation (primarily via deletion or suppression of PINK1 and parkin) causes the accumulation of dysfunctional mitochondria, which exhibit reduced mitochondrial membrane potential and increased ROS production. This condition lowers the threshold for mPTP opening, making mitophagy-deficient cells prone to undergo spontaneous mPT[116–118]. Confirming the important role of mitophagy in alleviating cellular stress resulting from mPT, CsA administration recovers mitochondrial alterations related to PINK1 deletion[117].

Regulation of stem cell fate. Several pieces of evidence link mPTP opening to cellular differentiation and the regulation of stem cell fate. During mouse cardiac development, especially at embryonic day 9.5 (the early phase of cardiac development in the mouse) when the tissue is enriched in stem/progenitor cells, mitochondria appear fragmented with disorganized cristae, a depressed mitochondrial membrane potential and elevated ROS production, suggesting frequent mPTP opening events[119]. By contrast, at embryonic day 13.5 (when the developing heart obtains the adult shape and is enriched in differentiated cells), this mitochondrial phenotype is suppressed. Emergence of the entire mitochondrial phenotype observed at embryonic day 9.5, was inhibited by CsA administration or CypD deletion. Most interestingly, pharmacological or genetic inhibition of the mPTP resulted in an acceleration of myocyte differentiation, while mPT induction delayed it[120]. Furthermore, the administration of pro-oxidants or antioxidants confirmed that mPT-induced ROS were responsible for the delay of the differentiation programme in cardiac stem cells[119]. Other investigations have demonstrated that mitophagy is required for myoblast differentiation in vitro[116] and for perinatal cardiac development[121], suggesting that the mPT and ROS promote stemness, whereas mitophagy can remodel the mitochondrial network, removing mitochondria with mPT to promote

Necrosis
A form of poorly regulated cell death characterized by energetic crisis and its consequent chaotic disruption of intracellular structures.

Mitophagy
The selective degradation of a mitochondrion via the lysosomal machinery.

PINK1–parkin system
A kinase/ubiquitinase system required for the tagging of outer mitochondrial membrane proteins, which in turn label mitochondria to be cleared via lysosomal degradation.

differentiation. Cardiac tissue appears phenotypically normal upon CypD loss in adult mice (*Ppif*$^{-/-}$)[12,106], but it displays reduced contractility under resting conditions and a higher propensity to develop hypertrophy and heart failure following pressure overload[35]. The impact of the mPTP on cardiomyocyte differentiation from pluripotent stem cells has also been demonstrated. In particular, the induction of differentiation to cardiomyocytes via dedicated media was favoured by the administration of CsA or its analogues[122].

These observations indicate that the cardiomyocytic differentiation programme specifically requires mitochondrial maturation and that mPTP opening opposes this process, favouring the maintenance of an undifferentiated phenotype. This principle appears to be a common trait for the maintenance of pluripotency. Indeed, it was observed that the generation of induced pluripotent stem cells also requires mPTP opening[123]. In particular, mouse embryonic fibroblasts transduced with the reprogramming factors *Sox2*, *Klf4*, *Oct4* (also known as *Pou5f1*) and *Myc* display features of spontaneous mPT, which can be reversed by exposure to CsA or knocking down *Ppif*. This event is associated with potentiation of the release of ROS and increase in miR-101c expression, which then promotes expression of plant homeodomain finger protein 8 (PHF8), a JmjC domain-containing protein that acts as a histone demethylase. PHF8 induction by the mPTP leads to demethylation of histone repressive marks and a reduction in their occupancies on the promoter regions of pluripotency genes, explaining the induction of pluripotency by mPT[123]. Furthermore, pharmacological desensitization of the mPTP favours the differentiation of induced pluripotent stem cells to endothelial cells[124]. It was also observed that mPT induces the release of α-ketoglutarate, a known regulator of stem cell fate decisions[125,126].

These observations are consistent with the most accepted model, which shows that poor mitochondrial maturation and low aerobic respiration efficiency favour stem cell maintenance and inhibit commitment in multiple models (for example, embryonic cells, adult cells or induced pluripotent stem cells)[125,126]. Furthermore, mitophagy has also been observed to participate in the maintenance of the immature mitochondrial phenotype[125,127]. Therefore, the emerging model is that spontaneous mPTP opening favours the immature mitochondrial state, which inhibits commitment of stem cells and supports their maintenance (FIG. 3a). Further investigation will be required to validate these mechanisms across stem cell types.

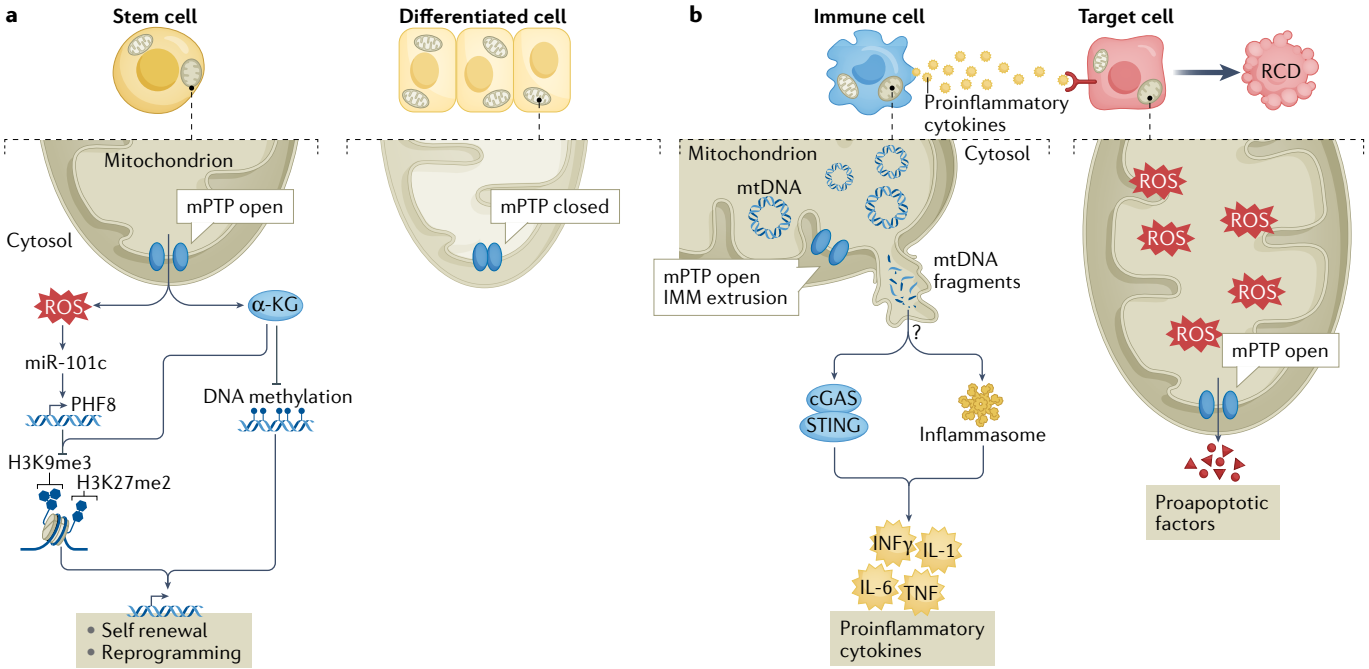
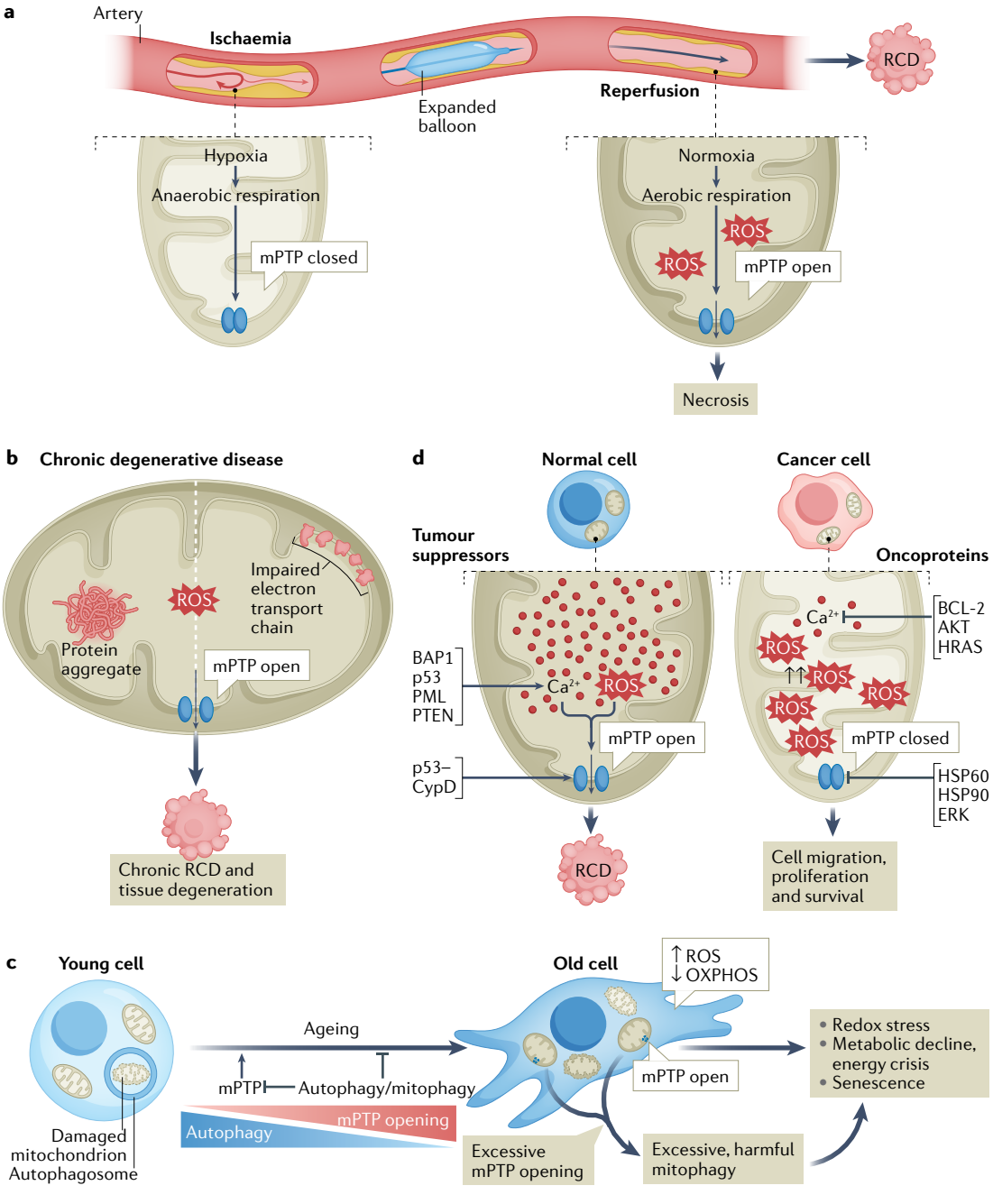


Fig. 3 | **Cellular roles of mPT. a** | Opening of the mitochondrial permeability transition pore (mPTP) can favour the maintenance of stem cells via mechanisms involving the release of reactive oxygen species (ROS) and α-ketoglutarate (α-KG) from mitochondria. ROS can activate the expression of histone demethylase plant homeodomain finger protein 8 (PHF8) by increasing the expression of its positive regulator, the microRNA miR-101C. PHF8, together with α-KG as its cofactor, downregulates inhibitory histone marks on promoters of pluripotency-associated genes, increasing their transcription. α-KG is also a cofactor for the DNA demethylation enzyme TET, promoting reduction of DNA methylation, which also inhibits gene expression (in this context, pluripotency-associated genes). These mechanisms support stem cell self-renewal and can favour cell reprogramming. Accordingly, a differentiated cell phenotype is associated with closure of the mPTP, and interference with mPTP opening can promote stem cell differentiation. **b** | In multiple models, including immune cells, mitochondrial permeability transition (mPT) favours the release of fragments of mitochondrial DNA (mtDNA), which engage the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway or activation of the inflammasome. Both pathways cause elevation of the levels of pro-inflammatory cytokines with the engagement of inflammatory response. Proinflammatory cytokines (for example, tumour necrosis factor (TNF), interferon-γ (IFNγ), interleukin-1 (IL-1) or IL-6) reaching a target cell (different cells types undergo this mechanism, for example, immune cells, hepatocytes and skeletal muscle cells) can elicit mPT via ROS production, ultimately leading to regulated cell death (RCD). The exact mechanism governing the release of the mtDNA is currently not known (question mark). H3K9me3, trimethylated histone H3 K9; H3K27me2, dimethylated histone H3 K27; IMM, inner mitochondrial membrane.

Initiation and potentiation of inflammation. The regulation of inflammation and mPTP opening are mutually linked. Genetic deletion of CypD reduces expression of pro-inflammatory cytokines in resting mouse aorta[128] and attenuates the inflammatory response induced by the exposure to bacterial products (such as lipopolysaccharide) in macrophages[129] or liver[130]. Also, the mPTP inhibitor Debio 025 inhibits spontaneous inflammation in a mouse model of Duchenne muscular dystrophy[131]. This suggests that mPT participates in the physiological regulation of the immune response, although a deeper characterization is required.

From the mechanistic perspective, prototypical pro-inflammatory cytokines, such as tumour necrosis factor (TNF), interferon-γ (IFNγ), interleukin-1 (IL-1) and IL-6, can induce mitochondrial dysfunction and RCD in a CsA-inhibitable manner[132–134]. Some reports indicate that the mPT is induced by cytokines via ROS production, although the existence of more regulated signalling downstream of cytokine receptors cannot be excluded[133,134].

In turn, inflammation can also be triggered by the mPTP. In multiple models, including immune cells, opening of the mPTP allows the release of mitochondrial DNA (mtDNA) fragments into the cytoplasm in response to irradiation, oxidative stress or exposure to lipopolysaccharide and extracellular ATP (acting as a danger signal informing of local cell damage)[130,135–137]. Considering the large dimensions of double-stranded DNA, it is unlikely that the mPTP directly mediates the crossing of mtDNA, and the mechanism of mtDNA passage through the IMM is still under investigation.



Damage-associated molecular pattern
A molecule released from a damaged or diseased cell able to stimulate a sterile immune or inflammatory response.

Inflammasome
A group of intracellular multimeric protein complexes that activate inflammatory caspase 1.

Amyotrophic lateral sclerosis
A progressive neurological disease that primarily affects the neurons responsible for controlling voluntary muscle movement.

Ischaemia
The deficient supply of blood to a tissue due to obstruction of the inflow of arterial blood.

Reperfusion injury
Type of tissue damage that is caused by the restoration of blood supply after a prolonged period of ischaemia.

Once in the cytosol, mtDNA functions as a damage-associated molecular pattern, activating cGMP–AMP synthase (cGAS), stimulator of interferon genes (STING) and inflammasome complexes, which, in turn, potentiate the production of pro-inflammatory cytokines. Which of the many inflammasome complexes are involved in mtDNA recognition is still debated[137,138].

The interaction between the mPTP and inflammation is important for understanding human diseases and may be responsible for the potentiation of inflammation in several conditions characterized by extensive inflammation. An example comes from recent observations in the context of amyotrophic lateral sclerosis. TDP43, a DNA/RNA-binding molecule that is often mutated in familial forms of amyotrophic lateral sclerosis, was recently proposed to mediate neurodegeneration via potentiation of the inflammatory response[139–141]. More recently, TDP43 was shown to localize to mitochondria to favour mtDNA release via the mPTP, cGAS–STING activation and progressive neurodegeneration in mouse models[142] (FIG. 3b).

Role of mPTP in diseases and ageing. In the previous section, we stressed the major cellular consequences of mPTP opening. These events can further develop to cause major alterations to tissues and organs, ultimately leading to pathological conditions.

Acute tissue failure in response to ischaemia–reperfusion. Ischaemia and consequent reperfusion injury are pathological manifestations in which the involvement of mPT has been robustly confirmed[14,143,144]. Ischaemia is associated with the adaptation to low oxygen level (hypoxia) in the tissue affected by the reduced blood supply. This adaptation causes a new, temporary equilibrium in the affected tissue that elevates the levels of mPTP inducers (Ca^{2+} and ROS), which are counterbalanced by a low pH and an increase in the ADP/ATP ratio. In addition, in in vitro models, the stabilization of hypoxia-inducible factor 1α (HIF1α) upregulates the mPTP desensitizer HKII[145,146], which protects the reoxygenation-induced mPTP[145] (see the following discussion). It is thought that in the ischaemic phase, mPT occurs only minimally, although a protective effect of CsA has been observed in some experimental models[147–149]. At the reperfusion stage, the restoration of partial pressure of O_2 recovers respiration, ATP synthesis and the activity of plasma membrane pumps, which re-equilibrate the intracellular pH. The inhibitory effect mediated by ADP and the acidic pH is weakened, and ROS production from multiple sources[150] is also stimulated by increased activity of the electron transport chain, causing the abrupt opening of the mPTP[151] (FIG. 4a).

One of the first and most compelling pieces of evidence involving mPT in ischaemia–reperfusion injury was provided with radioactive 2-deoxy-D-glucose[152]. To date, no mitochondrial transporters for 2-deoxy-D-glucose are known; therefore, its accumulation in the mitochondrial matrix is allowed only by the opening of the mPTP. Isolated dog hearts exposed to 2-deoxy-D-glucose and undergoing a cycle of ischaemia–reperfusion injury could accumulate this radioactive marker only in the reperfusion phase, and this effect could be inhibited by treatment with CsA[152]. This early evidence was later confirmed in several experimental models and tissues, including cardiac and skeletal muscle, brain, kidney, liver, lungs and testis[153–159]. In all these models, involvement of the mPTP was validated by the protective effect of CsA. Furthermore, the advent of knockout animal models for regulators of this phenomenon supported this concept. Indeed, protection of cardiac and neural tissues in terms of both tissue function and survival rates has been reported for *Ppif*$^{-/-}$ mice[12,106,160] and for animal models in which mitochondrial Ca^{2+} level was lowered or ablated, including cardiac-specific deletion of the plasma membrane Na^+–Ca^{2+} exchanger NCX (which is responsible for the intracellular elevation of Ca^{2+} level during ischaemia)[161], BCL-2 overexpression[162], cardiac-specific overexpression of the mitochondrial Na^+–Ca^{2+} exchanger (NCLX)[163] and pharmacological (by exposure to the mitochondrial Ca^{2+} uptake inhibitor Ru360) or genetic (knockout) blockade of mitochondrial calcium uniporter[164–166]. Recently, a mutation in the glycine-rich domain of ATP synthase subunit c, which is responsible for increased conductance in reconstituted c-ring and greater mPTP opening, was associated with increased mPTP opening and worse outcomes in patients with acute heart infarction[16,167]. CsA has significant mPTP-independent and cyclophilin A-dependent immunosuppressive activity[168]. Nonetheless, the use of CsA analogues lacking immunosuppressive activity, or CypD-independent mPTP inhibitors (TABLE 1), has displayed comparable protection in models of ischaemia–reperfusion injury[169–174]. At present CsA is the

◀ Fig. 4 | **Pathological consequences of mitochondrial permeability transition.** **a** | In ischaemic tissue, hypoxia sets the conditions to favour the closed state of the mitochondrial permeability transition pore (mPTP). At the reperfusion phase (for example, induced by counterpulsation of the intra-aortic balloon pump introduced into the blocked vessel), the restoration of normoxia elicits a rapid burst of reactive oxygen species (ROS), which triggers mPTP opening, ultimately leading to the induction of regulated cell death (RCD), mostly in the form of necrosis (owing to low supply of ATP in the postischaemic tissue) (FIG. 2b). **b** | In chronic diseases with degenerative evolution, the presence of detrimental mutations can lead to protein aggregates (for example, in neurogenerative diseases such as Parkinson disease or Alzheimer disease) or an impaired electron transport chain (for example, in mitochondrial diseases), which induces mitochondrial ROS production. This results in mPTP opening and RCD causing the chronic loss of postmitotic cells, ultimately leading to tissue degeneration. **c** | Ageing is characterized by progressive opening of the mPTP, accompanied by ROS production and inhibition of autophagic/mitophagic processes. The mPTP opening thus promotes cell decline and inhibits the anti-ageing effect of autophagy. Decline in mitophagy also promotes further mPTP opening, as mitochondria affected by the mPTP cannot be efficiently cleared by this quality control pathway (FIG. 2b). Such excessive mPTP opening can trigger harmful mitophagy, which might result in clearance of mitochondria without their replenishment, thereby resulting in a cellular energy crisis and contributing to cell function decline. **d** | The suppression of mPTP opening is proposed to participate in tumour progression. In normal cells, ROS production and mitochondrial Ca^{2+} cooperate to induce mPTP opening. This mechanism is favoured by the activity of some oncosuppressor proteins (for example, BAP1, p53, PML and PTEN). The tumour suppressor p53 is also reported to favour the direct opening of the mPTP via the interaction with cyclophilin D (CypD). In tumour cells, loss of oncosuppressor protein and the activation of oncoproteins (for example, BCL-2, AKT and HRAS) impairs Ca^{2+} signalling, disfavouring mPTP opening and RCD induction, even despite an elevation of ROS levels (independent of mPTP), which generally, in cancer, promote cell migration, proliferation and survival. Also, some tumour cells display increased levels of HSP60, HSP90 or ERK signalling, which have an inhibitory effect on the mPTP. OXPHOS, oxidative phosphorylation.

only established mPTP inhibitor that has entered clinical trials to test its efficacy in treatment of ischaemia–reperfusion injury. However, the results obtained from the trials indicate that CsA administration did not provide any measurable clinical benefit when administered at the time of reperfusion. This might be due to the limited knowledge of mPTP dynamics in the human disease, the pharmacokinetic properties of CsA as well as the presence of confounding factors in the selected cohorts (for example, the presence of co-morbidities or concurrent medications), still leaving the mPTP as a potential target for the treatment of this disease[175].

Involvement of the mPTP is also proposed for acute tissue failure events that occur at normal partial pressure of O_2 (therefore not ischaemic), which is particularly true for acute kidney injury. Indeed, extended necrotization of kidney tissue is frequently observed because of exposure to drugs (for example, non-steroidal anti-inflammatory drugs or chemotherapy) and crystal-induced kidney injury[176,177]. The cause of this tissue damage, which resembles reperfusion injury, is not yet fully understood, although it is proposed to involve excess ROS production, which ultimately triggers mPT[178]. In agreement with this possibility, inhibition of GSK3β impairs mPT induction in models of drug-induced nephrotoxicity[179,180]. Substantial evidence based on knockout animal models indicates that in acute kidney injury, mPT mediates tissue damage by the induction of a subtype of RCD named 'necroptosis'[176,177,181].

Diseases with chronic manifestations. A role for mPT has also been proposed for conditions with a chronic and degenerative evolution, which are often associated with ageing. Such is the case for Alzheimer disease[182,183], Parkinson disease (and related subcategories)[184,185], cardiovascular diseases such as heart failure[35,143,186] and atherosclerosis, and overt genetic conditions such as mitochondrial disorders (related to mtDNA mutations) and other conditions (for example, multiple sclerosis, Ullrich congenital muscular dystrophy and dominant optic atrophy)[183,187–192].

The rationale for the involvement of mPT in these various diseases is that a disease-associated perturbation of the cellular environment (for example, the presence of aggregates or defective function of the respiratory chain) directly or indirectly causes the elevation of intracellular ROS production, mild dissipation of mitochondrial membrane potential and, less frequently, elevation of intracellular Ca^{2+} level. These phenomena will increase the frequency of spontaneous mPT, negatively impacting on cellular bioenergetics, which reduces cell viability and eventually causes excessive RCD. This sequence of events ultimately causes major functional impairment of the tissue and eventually organ failure (FIG. 4b).

For most of the conditions mentioned, alterations of mitochondrial function and different mPTP opening thresholds have been reported in samples obtained from patients[183,187,188] as well as in related animal models[35,189–191]. The leading cause of mitochondrial alteration is proposed to vary among different conditions. For example, for Alzheimer disease and Parkinson disease, the presence of protein aggregates can directly affect mitochondria or impair the mitophagy process[183,189,191,193–200]. Again, for some of the genetic disorders mentioned, the mutation causes disproportionate expression of respiratory complex subunits and inefficient assembly or direct impairment of their activity. For multifactorial diseases (for example, heart failure or multiple sclerosis), mPT activation mechanisms are still unclear[14]. Additionally, for many genetic animal models of these conditions, a partial recovery of tissue function and a reduction in RCD were obtained by deletion of *Ppif*, with prolonged administration of mPTP inhibitors (for example, CsA, the 4-aminobenzenesulfonamide derivative C-9 and GNX-4728) or removal of external Ca^{2+} (REFS[183,185,201–207]).

Ageing. Multiple lines of evidence have shown that mPT is associated with ageing. In particular, the mPTP opening threshold is lowered in mitochondria from old mice versus young mice[208–210]. Additionally, the spontaneous flickering of the mPTP was associated with ageing in *Caenorhabditis elegans*[211].

The reason for the increased probability of mPTP opening in aged tissues remains unknown. Differences in the mPTP between young mice and old mice are dramatically attenuated by the administration of CsA, suggesting that the driving alteration lies in the mechanism controlling the mPTP threshold rather than structural alteration of its core. Consistent with this possibility, aged tissues are often reported to have increased levels of pro-oxidants[212], which could sensitize the mPTP to induction.

The mPT in aged cells could contribute to detrimental tissue degeneration via excessive activation of RCD or increased ROS production (supporting the free radical theory of ageing). It has also been proposed that prolonged mPTP opening participates in ageing-dependent depletion of cellular NAD^+. This molecule opposes cellular ageing, partly via sirtuin activity[213]. The mPT favours NAD^+ redistribution from the mitochondrial matrix to the cytosol, where it is hydrolysed by the NAD^+-consuming enzymes CD38 and PARP1 (REFS[92,214]). In addition, in the ageing mouse brain, the CypD–OSCP interaction is favoured, resulting in sensitized mPTP opening[215]. Interestingly, *Ppif* deletion in one allele increased the mouse lifespan, in contrast to complete knockout, suggesting that while mild mPTP inhibition could be beneficial in acting to slow ageing, its complete inhibition might be detrimental, possibly because of the loss of its physiological roles (described in the previous section)[216].

In contrast to mPT, the mitophagy process appears to be under-represented in aged tissue[217–219]. Mitophagy (and more generally autophagy) opposes ageing, and multiple strategies that favour mitophagy/autophagy have been proven to increase lifespan in multiple systems[220–222]. Recent evidence indicates that the anti-ageing effect of autophagy appears to be dependent on mPTP inhibition. Mechanistic target of rapamycin (mTOR) complex 2 (mTORC2) inhibits autophagy via serum/glucocorticoid-regulated kinase 1 (SGK1). SGK1 phosphorylates VDAC, favouring its ubiquitination and degradation[223,224], finally desensitizing mPTP opening[224]. Genetic interference with SGK-1 in *C. elegans* causes

Necroptosis
A form of regulated cell death initiated by death receptors and dependent on RIPK1, RIPK3 and MLKL.

Ullrich congenital muscular dystrophy
A rare hereditary muscle condition characterized by abnormalities in collagen type VI, resulting in impaired progressive muscle weakness.

Free radical theory of ageing
A theory of ageing proposing that organisms age because they accumulate oxidative damage.

NAD^+
A molecule that carries reduced equivalents between enzymes to run redox reactions. Decline in its level is associated with ageing and ageing-related mitochondrial dysfunction.

VDAC accumulation, mPTP opening and mitochondrial ROS production. Curiously, these changes promote increased autophagy/mitophagy, finally leading to a dramatic reduction in lifespan. Inhibition of autophagy or the mPTP in SGK-1-deficient *C. elegans* restores lifespan[224]. These data suggest that mitophagy could help prevent detrimental mPTP opening during ageing, thereby preserving cell function, and that ageing-associated decline in autophagy leads to cell dysfunction, at least partly due to harmful effects of mPT as described above. Interestingly, excessive mPTP opening seems to promote autophagy with harmful effects on lifespan. It is proposed that this is a rather unexpected form of autophagy, leading to non-specific clearance of mitochondria and failure to replace those mitochondria with normal, healthy organelles, which ultimately accelerates cell dysfunction. This intriguing possibility could provide a significant advance in understanding the relationship between autophagy and longevity and deserves further investigation (FIG. 4c).

Overall, pathological mPTP opening appears to be a hallmark of ageing, and further investigation will inform on the possibilities of its targeting for ageing amelioration.

Desensitization of the mPTP in malignancies. As the mPTP is a mediator of RCD, it was long hypothesized that suppression of the mPTP could be a feature of neoplastic cells. To date, there are no reports that confirm or refute this hypothesis, as mPTP opening could be easily achieved in tumour cells in vitro.

In addition, ROS production is generally considered augmented in cancer cells, favouring tumour cell proliferation and migration[225,226]. This suggests that mPTP opening could be stimulated rather than inhibited in neoplastic cells. Considering the dramatic selective pressure to which tumour cells are exposed, it could be speculated that another mechanism intervenes to desensitize mPTP opening and permit protumorigenic ROS production without engaging RCD.

Several oncogenes whose activity is associated with suppression of RCD have been demonstrated to negatively impact mitochondrial Ca^{2+} uptake (for example, AKT, BCL-2 and HRAS)[227–229]. Concordantly, the product of some tumour suppressors can potentiate RCD by favouring mitochondrial Ca^{2+} uptake (for example, BAP1, p53, PTEN and PML)[230–234]. This indicates that tumour-promoting activity is at least partially mediated by reducing the availability of mitochondrial Ca^{2+} for the triggering of mPT and consequent RCD. Tumour suppressors counteract this mechanism by augmenting mitochondrial Ca^{2+} availability (FIG. 4d).

Cancer cells can also inhibit CypD via potentiation of ERK activity[72] or overexpression of the chaperones HSP90 and HSP60 (REFS[64–66]). Interestingly the master tumour suppressor p53 can induce mPTP opening via interaction with CypD[235] and ATP synthase stability via interaction with OSCP[236]. Finally, p53 is known to interact with TRAP1 and HSP90 (REFS[237,238]), and it is proposed that the p53 inactivation, which is extremely frequent in neoplastic lesions, can further contribute to mPTP desensitization via this chaperone network[239].

Conclusions and perspective

mPT has attracted considerable attention owing to its involvement in multiple cellular states. Indeed, it impacts mitochondrial physiology, cellular energy metabolism and RCD activation, making it an attractive target for regulating multiple physiological and pathological conditions. The use of *Ppif*$^{-/-}$ mice is an essential approach for investigating the mPTP; such investigations have revealed the participation of CypD in unexpected phenomena. Nevertheless, a major challenge in mPTP investigations is that apart from CypD, there is no consensus on its structure. Fortunately, a novel model involving ATP synthase has been proposed (BOX 1), and experimental confirmation is ongoing. Nevertheless, there are major uncertainties about how the non-specific pore is formed by this complex molecular machine, and how it becomes sensitive to Ca^{2+} and ROS as well as what mechanisms of voltage sensing are involved; significant effort must be invested to confirm recent observations on the structural reorganization of ATP synthase during mPTP opening and add details to the model. Such efforts would also benefit from the use of standardized procedures to measure mPTP activity from isolated mitochondria or living cells (Supplementary Box 1). Inhibitors of CypD (as well as its knockout) have demonstrated the merit of efforts to investigate the mPTP in animal models. Experimental models recapitulating multiple human diseases have now shown that mPTP desensitization can lead to amelioration of disease. Nevertheless, no drug targeting the mPTP has had positive results in completed clinical trials, which might be due to different factors. First, the drugs tested so far had relatively poor pharmacokinetic properties and multiple side effects compared with newer and more refined mPTP inhibitors. Second, the modality of delivery may not have been optimal, and it is unknown whether the drug reached its target. Third, the drugs tested thus far target regulators of the mPTP rather than its core, possibly diminishing their efficacy.

Therefore, although the activity of the mPTP was identified more than 40 years ago, the molecular aspects of its function remain elusive. The improved cryogenic electron microscopy technology will be instrumental in describing the ATP synthase structure during genetic and pharmacological modulation of the mPTP. This technology will significantly improve the current pore formation model and, together with data from ATP synthase mutants already available, will allow the generation of a better experimental model to investigate mPT-related phenomena. The ideal model will include mutants in ATP synthase subunits and ANT capable of affecting mPTP formation without measurably affecting mitochondrial ATP homeostasis. Two mutants have already been proposed to affect mPTP formation with no alteration of ATP levels, located respectively in subunit c and β-subunit of ATP synthase, although mouse models are still not available. These models will permit the identification of low-conductance and high-conductance mPTP-dependent phenomena and inform on CypD functions not related to mPT. Eventually, these advances will provide a clearer picture of mPT-related diseases and better pharmacological strategies for their modulation.

Published online: 08 December 2021

1. Galluzzi, L. et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* **25**, 486–541 (2018).
2. Nakamura-Ishizu, A., Ito, K. & Suda, T. Hematopoietic stem cell metabolism during development and aging. *Dev. Cell* **54**, 239–255 (2020).
3. Giorgi, C., Marchi, S. & Pinton, P. The machineries, regulation and cellular functions of mitochondrial calcium. *Nat. Rev. Mol. Cell Biol.* **19**, 713–730 (2018).
4. Zhang, F., Zhang, L., Qi, Y. & Xu, H. Mitochondrial cAMP signaling. *Cell Mol. Life Sci.* **73**, 4577–4590 (2016).
5. Sies, H. & Jones, D. P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **21**, 363–383 (2020).
6. Hansen, K. G. & Herrmann, J. M. Transport of proteins into mitochondria. *Protein J.* **38**, 330–342 (2019).
7. Giacomello, M., Pyakurel, A., Glytsou, C. & Scorrano, L. The cell biology of mitochondrial membrane dynamics. *Nat. Rev. Mol. Cell Biol.* **21**, 204–224 (2020).
8. Rosencrans, W. M., Rajendran, M., Bezrukov, S. M. & Rostovtseva, T. K. VDAC regulation of mitochondrial calcium flux: from channel biophysics to disease. *Cell Calcium* **94**, 102356 (2021).
9. Haworth, R. A. & Hunter, D. R. The Ca^{2+}-induced membrane transition in mitochondria. II. Nature of the Ca^{2+} trigger site. *Arch. Biochem. Biophys.* **195**, 460–467 (1979).
10. Hunter, D. R. & Haworth, R. A. The Ca^{2+}-induced membrane transition in mitochondria. III. Transitional Ca^{2+} release. *Arch. Biochem. Biophys.* **195**, 468–477 (1979).
11. Hunter, D. R. & Haworth, R. A. The Ca^{2+}-induced membrane transition in mitochondria. I. The protective mechanisms. *Arch. Biochem. Biophys.* **195**, 453–459 (1979).
This work, together with workfrom Haworth and Hunter (1979) and Hunter and Haworth (1979), formallydefines the properties of mPT from isolated mitochondria.
12. Baines, C. P. et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**, 658–662 (2005).
13. Giorgio, V. et al. Cyclophilin D in mitochondrial pathophysiology. *Biochim. Biophys. Acta* **1797**, 1113–1118 (2010).
14. Bonora, M. et al. Physiopathology of the permeability transition pore: molecular mechanisms in human pathology. *Biomolecules* **10**, 998 (2020).
15. Spikes, T. E., Montgomery, M. G. & Walker, J. E. Structure of the dimeric ATP synthase from bovine mitochondria. *Proc. Natl Acad. Sci. USA* **117**, 23519–23526 (2020).
16. Alavian, K. N. et al. An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. *Proc. Natl Acad. Sci. USA* **111**, 10580–10585 (2014).
17. Giorgio, V. et al. Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc. Natl Acad. Sci. USA* **110**, 5887–5892 (2013).
Together with Alavian et al. (2014), this study is the firstto demonstrate that purified ATP synthase can generate mPTP-like currents inartificial membrane systems.
18. Urbani, A. et al. Purified F-ATP synthase forms a Ca^{2+}-dependent high-conductance channel matching the mitochondrial permeability transition pore. *Nat. Commun.* **10**, 4341 (2019).
19. Mnatsakanyan, N. et al. A mitochondrial megachannel resides in monomeric F1FO ATP synthase. *Nat. Commun.* **10**, 5823 (2019).
20. Bonora, M. et al. Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle* **12**, 674–683 (2013).
This work proposes for the first time the involvement of ATP synthase in mPTP composition.
21. Bonora, M. et al. Mitochondrial permeability transition involves dissociation of F1FO ATP synthase dimers and C-ring conformation. *EMBO Rep.* **18**, 1077–1089 (2017).
22. Galber, C. et al. The f subunit of human ATP synthase is essential for normal mitochondrial morphology and permeability transition. *Cell Rep.* **35**, 109111 (2021).
23. Carraro, M. et al. Channel formation by yeast F-ATP synthase and the role of dimerization in the mitochondrial permeability transition. *J. Biol. Chem.* **289**, 15980–15985 (2014).
24. Carraro, M. et al. The unique cysteine of F-ATP synthase OSCP subunit participates in modulation of the permeability transition pore. *Cell Rep.* **32**, 108095 (2020).
25. Giorgio, V. et al. Ca^{2+} binding to F-ATP synthase beta subunit triggers the mitochondrial permeability transition. *EMBO Rep.* **18**, 1065–1076 (2017).
26. Antoniel, M. et al. The unique histidine in OSCP subunit of F-ATP synthase mediates inhibition of the permeability transition pore by acidic pH. *EMBO Rep.* **19**, 257–268 (2018).
27. Guo, L. et al. Arginine 107 of yeast ATP synthase subunit g mediates sensitivity of the mitochondrial permeability transition to phenylglyoxal. *J. Biol. Chem.* **293**, 14632–14645 (2018).
28. Ichas, F. & Mazat, J. P. From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochim. Biophys. Acta* **1366**, 33–50 (1998).
29. Szabo, I. & Zoratti, M. The mitochondrial megachannel is the permeability transition pore. *J. Bioenerg. Biomembr.* **24**, 111–117 (1992).
This study describes for the first time the electrophysiological properties of the mPTP.
30. Savage, M. K. & Reed, D. J. Release of mitochondrial glutathione and calcium by a cyclosporin A-sensitive mechanism occurs without large amplitude swelling. *Arch. Biochem. Biophys.* **315**, 142–152 (1994).
31. Neginskaya, M. A. et al. ATP synthase c-subunit-deficient mitochondria have a small cyclosporine a-sensitive channel, but lack the permeability transition pore. *Cell Rep.* **26**, 11–17 e12 (2019).
This work describes an mPTP-like low-conductance current attributable to ANT in cells deprived of the c-ring of ATP synthase.
32. Szabo, I. & Zoratti, M. The mitochondrial permeability transition pore may comprise VDAC molecules. I. Binary structure and voltage dependence of the pore. *FEBS Lett.* **330**, 201–205 (1993).
33. Boyman, L. et al. Dynamics of the mitochondrial permeability transition pore: transient and permanent opening events. *Arch. Biochem. Biophys.* **666**, 31–39 (2019).
34. Petronilli, V. et al. Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence. *Biophys. J.* **76**, 725–734 (1999).
35. Elrod, J. W. et al. Cyclophilin D controls mitochondrial pore-dependent Ca^{2+} exchange, metabolic flexibility, and propensity for heart failure in mice. *J. Clin. Invest.* **120**, 3680–3687 (2010).
This study describes cellular metabolism in *Ppif*$^{-/-}$ mouse models, providing the first evidence for a role of the mPTP in the physiological regulation of metabolism.
36. Giorgio, V., Guo, L., Bassot, C., Petronilli, V. & Bernardi, P. Calcium and regulation of the mitochondrial permeability transition. *Cell Calcium* **70**, 56–63 (2018).
37. Karch, J. et al. Inhibition of mitochondrial permeability transition by deletion of the ANT family and CypD. *Sci. Adv.* **5**, eaaw4597 (2019).
The complete knockdown of all mouse ANT isoforms confirms the role of ANT in mPT and provides additional evidence for two independent pores.
38. Carrer, A. et al. Defining the molecular mechanisms of the mitochondrial permeability transition through genetic manipulation of F-ATP synthase. *Nat. Commun.* **12**, 4835 (2021).
39. Pinke, G., Zhou, L. & Sazanov, L. A. Cryo-EM structure of the entire mammalian F-type ATP synthase. *Nat. Struct. Mol. Biol.* **27**, 1077–1085 (2020).
This study describes for the first time the structure of ATP synthase in conditions of mPTP opening, providing solid confirmation of the c-ring model.
40. Algieri, C. et al. Mitochondrial Ca^{2+} -activated F1FO-ATPase hydrolyzes ATP and promotes the permeability transition pore. *Ann. NY Acad. Sci.* **1457**, 142–157 (2019).
41. Carroll, J., He, J., Ding, S., Fearnley, I. M. & Walker, J. E. Persistence of the permeability transition pore in human mitochondria devoid of an assembled ATP synthase. *Proc. Natl Acad. Sci. USA* **116**, 12816–12821 (2019).
42. He, J. et al. Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl Acad. Sci. USA* **114**, 3409–3414 (2017).
43. Zhou, W., Marinelli, F., Nief, C. & Faraldo-Gomez, J. D. Atomistic simulations indicate the c-subunit ring of the F1Fo ATP synthase is not the mitochondrial permeability transition pore. *eLife* **6**, e23781 (2017).
44. Gerle, C. Mitochondrial F-ATP synthase as the permeability transition pore. *Pharmacol. Res.* **160**, 105081 (2020).
45. Hunter, D. R., Haworth, R. A. & Southard, J. H. Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J. Biol. Chem.* **251**, 5069–5077 (1976).
46. Kokoszka, J. E. et al. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**, 461–465 (2004).
47. Petronilli, V., Nicolli, A., Costantini, P., Colonna, R. & Bernardi, P. Regulation of the permeability transition pore, a voltage-dependent mitochondrial channel inhibited by cyclosporin A. *Biochim. Biophys. Acta* **1187**, 255–259 (1994).
48. Linder, M. D., Morkunaite-Haimi, S., Kinnunen, P. K., Bernardi, P. & Eriksson, O. Ligand-selective modulation of the permeability transition pore by arginine modification. Opposing effects of p-hydroxyphenylglyoxal and phenylglyoxal. *J. Biol. Chem.* **277**, 937–942 (2002).
49. Zorov, D. B., Kinnally, K. W., Perini, S. & Tedeschi, H. Multiple conductance levels in rat heart inner mitochondrial membranes studied by patch clamping. *Biochim. Biophys. Acta* **1105**, 263–270 (1992).
50. Halestrap, A. P., Connern, C. P., Griffiths, E. J. & Kerr, P. M. Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischaemia/reperfusion injury. *Mol. Cell Biochem.* **174**, 167–172 (1997).
This work identifies CypD as the target of CsA in mPTP inhibition.
51. Kanno, T. et al. Oxidative stress underlies the mechanism for Ca^{2+}-induced permeability transition of mitochondria. *Free Radic. Res.* **38**, 27–35 (2004).
52. Kowaltowski, A. J., Netto, L. E. & Vercesi, A. E. The thiol-specific antioxidant enzyme prevents mitochondrial permeability transition. Evidence for the participation of reactive oxygen species in this mechanism. *J. Biol. Chem.* **273**, 12766–12769 (1998).
53. Costantini, P., Chernyak, B. V., Petronilli, V. & Bernardi, P. Modulation of the mitochondrial permeability transition pore by pyridine nucleotides and dithiol oxidation at two separate sites. *J. Biol. Chem.* **271**, 6746–6751 (1996).
54. Halestrap, A. P., Woodfield, K. Y. & Connern, C. P. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *J. Biol. Chem.* **272**, 3346–3354 (1997).
55. Kaludercic, N. & Giorgio, V. The dual function of reactive oxygen/nitrogen species in bioenergetics and cell death: the role of ATP synthase. *Oxid. Med. Cell Longev.* **2016**, 3869610 (2016).
56. Vercesi, A. E., Kowaltowski, A. J., Grijalba, M. T., Meinicke, A. R. & Castilho, R. F. The role of reactive oxygen species in mitochondrial permeability transition. *Biosci. Rep.* **17**, 43–52 (1997).
57. Kowaltowski, A. J., Castilho, R. F. & Vercesi, A. E. Opening of the mitochondrial permeability transition pore by uncoupling or inorganic phosphate in the presence of Ca^{2+} is dependent on mitochondrial-generated reactive oxygen species. *FEBS Lett.* **378**, 150–152 (1996).
58. Kowaltowski, A. J., Vercesi, A. E., Rhee, S. G. & Netto, L. E. Catalases and thioredoxin peroxidase protect Saccharomyces cerevisiae against Ca^{2+}-induced mitochondrial membrane permeabilization and cell death. *FEBS Lett.* **473**, 177–182 (2000).
59. Samovski, D., Kalderon, B., Yehuda-Shnaidman, E. & Bar-Tana, J. Gating of the mitochondrial permeability transition pore by long chain fatty acyl analogs in vivo. *J. Biol. Chem.* **285**, 6879–6890 (2010).
60. Betaneli, V., Petrov, E. P. & Schwille, P. The role of lipids in VDAC oligomerization. *Biophys. J.* **102**, 523–531 (2012).
61. Wieckowski, M. R., Brdiczka, D. & Wojtczak, L. Long-chain fatty acids promote opening of the reconstituted mitochondrial permeability transition pore. *FEBS Lett.* **484**, 61–64 (2000).
62. Furuno, T. et al. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. *Biochem. Pharmacol.* **62**, 1037–1046 (2001).

63. Oyanagi, E., Yano, H., Uchida, M., Utsumi, K. & Sasaki, J. Protective action of L-carnitine on cardiac mitochondrial function and structure against fatty acid stress. *Biochem. Biophys. Res. Commun.* **412**, 61–67 (2011).
64. Kang, B. H. et al. Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. *Cell* **131**, 257–270 (2007).
65. Ghosh, J. C., Siegelin, M. D., Dohi, T. & Altieri, D. C. Heat shock protein 60 regulation of the mitochondrial permeability transition pore in tumor cells. *Cancer Res.* **70**, 8988–8993 (2010).
66. Sinha, D. & D'Silva, P. Chaperoning mitochondrial permeability transition: regulation of transition pore complex by a J-protein, DnaJC15. *Cell Death Dis.* **5**, e1101 (2014).
67. Margineantu, D. H., Emerson, C. B., Diaz, D. & Hockenbery, D. M. Hsp90 inhibition decreases mitochondrial protein turnover. *PLoS ONE* **2**, e1066 (2007).
68. Hafner, A. V. et al. Regulation of the mPTP by SIRT3-mediated deacetylation of CypD at lysine 166 suppresses age-related cardiac hypertrophy. *Aging* **2**, 914–923 (2010).
69. Bochaton, T. et al. Inhibition of myocardial reperfusion injury by ischemic postconditioning requires sirtuin 3-mediated deacetylation of cyclophilin D. *J. Mol. Cell Cardiol.* **84**, 61–69 (2015).
70. Yang, W. et al. Mitochondrial sirtuin network reveals dynamic SIRT3-dependent deacetylation in response to membrane depolarization. *Cell* **167**, 985–1000.e21 (2016).
71. Lee, C. F. et al. Normalization of NAD^+ redox balance as a therapy for heart failure. *Circulation* **134** (2016).
72. Rasola, A. et al. Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition. *Proc. Natl Acad. Sci. USA* **107**, 726–731 (2010).
This study demonstrates for the first time that signalling pathways can have a direct effect on mPTP activity.
73. Hurst, S. et al. Phosphorylation of cyclophilin D at serine 191 regulates mitochondrial permeability transition pore opening and cell death after ischemia-reperfusion. *Cell Death Dis.* **11**, 661 (2020).
74. Nishihara, M. et al. Modulation of the mitochondrial permeability transition pore complex in GSK-3beta-mediated myocardial protection. *J. Mol. Cell Cardiol.* **43**, 564–570 (2007).
75. Wang, S. et al. Mitochondrial PKC-epsilon deficiency promotes I/R-mediated myocardial injury via GSK3beta-dependent mitochondrial permeability transition pore opening. *J. Cell Mol. Med.* **21**, 2009–2021 (2017).
76. Tanno, M. et al. Translocation of glycogen synthase kinase-3beta (GSK-3beta), a trigger of permeability transition, is kinase activity-dependent and mediated by interaction with voltage-dependent anion channel 2 (VDAC2). *J. Biol. Chem.* **289**, 29285–29296 (2014).
77. Gomez, L. et al. The SR/ER-mitochondria calcium crosstalk is regulated by GSK3beta during reperfusion injury. *Cell Death Differ.* **23**, 313–322 (2016).
78. Yang, K. et al. The key roles of GSK-3beta in regulating mitochondrial activity. *Cell Physiol. Biochem.* **44**, 1445–1459 (2017).
79. Ghosh, J. C. et al. Adaptive mitochondrial reprogramming and resistance to PI3K therapy. *J. Natl Cancer Inst.* **107**, dju502 (2015).
80. Whittington, H. J. et al. Over-expression of mitochondrial creatine kinase in the murine heart improves functional recovery and protects against injury following ischaemia-reperfusion. *Cardiovasc. Res.* **114**, 858–869 (2018).
81. Datler, C. et al. CKMT1 regulates the mitochondrial permeability transition pore in a process that provides evidence for alternative forms of the complex. *J. Cell Sci.* **127**, 1816–1828 (2014).
82. Woldetsadik, A. D., Vogel, M. C., Rabeh, W. M. & Magzoub, M. Hexokinase II-derived cell-penetrating peptide targets mitochondria and triggers apoptosis in cancer cells. *FASEB J.* **31**, 2168–2184 (2017).
83. Baines, C. P. et al. Protein kinase Cepsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. *Circ. Res.* **92**, 873–880 (2003).
84. Pediaditakis, P. et al. Inhibition of the mitochondrial permeability transition by protein kinase A in rat liver mitochondria and hepatocytes. *Biochem. J.* **431**, 411–421 (2010).
85. Karch, J. et al. Bax and Bak function as the outer membrane component of the mitochondrial permeability pore in regulating necrotic cell death in mice. *eLife* **2**, e00772 (2013).
This study reveals that OMM remodelling during mPT-induced mitochondrial swelling is dependent on BAX and BAK.
86. Whelan, R. S. et al. Bax regulates primary necrosis through mitochondrial dynamics. *Proc. Natl Acad. Sci. USA* **109**, 6566–6571 (2012).
87. Roy, S. S. et al. Bad targets the permeability transition pore independent of Bax or Bak to switch between Ca^{2+}-dependent cell survival and death. *Mol. Cell* **33**, 377–388 (2009).
88. Alavian, K. N. et al. Bcl-xL regulates metabolic efficiency of neurons through interaction with the mitochondrial F1FO ATP synthase. *Nat. Cell Biol.* **13**, 1224–1233 (2011).
89. Park, H. A. et al. Inhibition of Bcl-xL prevents pro-death actions of DeltaN-Bcl-xL at the mitochondrial inner membrane during glutamate excitotoxicity. *Cell Death Differ.* **24**, 1963–1974 (2017).
90. Zorov, D. B., Filburn, C. R., Klotz, L. O., Zweier, J. L. & Sollott, S. J. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J. Exp. Med.* **192**, 1001–1014 (2000).
91. Dumas, J. F. et al. Effect of transient and permanent permeability transition pore opening on NAD(P)H localization in intact cells. *J. Biol. Chem.* **284**, 15117–15125 (2009).
92. Di Lisa, F., Menabo, R., Canton, M., Barile, M. & Bernardi, P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic NAD^+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. *J. Biol. Chem.* **276**, 2571–2575 (2001).
93. Jang, S. et al. Elucidating mitochondrial electron transport chain supercomplexes in the heart during ischemia-reperfusion. *Antioxid. Redox Signal.* **27**, 57–69 (2017).
94. Batandier, C., Leverve, X. & Fontaine, E. Opening of the mitochondrial permeability transition pore induces reactive oxygen species production at the level of the respiratory chain complex I. *J. Biol. Chem.* **279**, 17197–17204 (2004).
95. McArthur, K. et al. BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science* **359**, eaao6047 (2018).
96. Tavecchio, M., Lisanti, S., Bennett, M. J., Languino, L. R. & Altieri, D. C. Deletion of cyclophilin D impairs beta-oxidation and promotes glucose metabolism. *Sci. Rep.* **5**, 15981 (2015).
97. Taddeo, E. P. et al. Opening of the mitochondrial permeability transition pore links mitochondrial dysfunction to insulin resistance in skeletal muscle. *Mol. Metab.* **3**, 124–134 (2014).
98. Laker, R. C. et al. The mitochondrial permeability transition pore regulator cyclophilin D exhibits tissue-specific control of metabolic homeostasis. *PLoS ONE* **11**, e0167910 (2016).
99. Menazza, S. et al. $CypD^{-/-}$ hearts have altered levels of proteins involved in Krebs cycle, branch chain amino acid degradation and pyruvate metabolism. *J. Mol. Cell Cardiol.* **56**, 81–90 (2013).
100. Wang, X. et al. Cyclophilin D deficiency attenuates mitochondrial perturbation and ameliorates hepatic steatosis. *Hepatology* **68**, 62–77 (2018).
101. Zorov, D. B., Juhaszova, M. & Sollott, S. J. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* **94**, 909–950 (2014).
102. Bonora, M. et al. Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition. *Oncogene* **34**, 1608 (2015).
103. Bauer, T. M. & Murphy, E. Role of mitochondrial calcium and the permeability transition pore in regulating cell death. *Circ. Res.* **126**, 280–293 (2020).
104. Borutaite, V., Jekabsone, A., Morkuniene, R. & Brown, G. C. Inhibition of mitochondrial permeability transition prevents mitochondrial dysfunction, cytochrome c release and apoptosis induced by heart ischemia. *J. Mol. Cell Cardiol.* **35**, 357–366 (2003).
105. Precht, T. A. et al. The permeability transition pore triggers Bax translocation to mitochondria during neuronal apoptosis. *Cell Death Differ.* **12**, 255–265 (2005).
106. Nakagawa, T. et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* **434**, 652–658 (2005).
107. Kim, J. S., He, L. & Lemasters, J. J. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. *Biochem. Biophys. Res. Commun.* **304**, 463–470 (2003).
108. Rodriguez-Enriquez, S., Kim, I., Currin, R. T. & Lemasters, J. J. Tracker dyes to probe mitochondrial autophagy (mitophagy) in rat hepatocytes. *Autophagy* **2**, 39–46 (2006).
This work describes for the first time that mPTP opening can trigger mitophagy.
109. Elmore, S. P., Qian, T., Grissom, S. F. & Lemasters, J. J. The mitochondrial permeability transition initiates autophagy in rat hepatocytes. *FASEB J.* **15**, 2286–2287 (2001).
110. Carreira, R. S., Lee, Y., Ghochani, M., Gustafsson, A. B. & Gottlieb, R. A. Cyclophilin D is required for mitochondrial removal by autophagy in cardiac cells. *Autophagy* **6**, 462–472 (2010).
111. Rimessi, A. et al. Perturbed mitochondrial Ca^{2+} signals as causes or consequences of mitophagy induction. *Autophagy* **9**, 1677–1686 (2013).
112. Medina, D. L. & Ballabio, A. Lysosomal calcium regulates autophagy. *Autophagy* **11**, 970–971 (2015).
113. Palikaras, K., Lionaki, E. & Tavernarakis, N. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. *Nat. Cell Biol.* **20**, 1013–1022 (2018).
114. Song, M., Mihara, K., Chen, Y., Scorrano, L. & Dorn, G. W. II Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. *Cell Metab.* **21**, 273–286 (2015).
115. Vives-Bauza, C. et al. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc. Natl Acad. Sci. USA* **107**, 378–383 (2010).
116. Baechler, B. L., Bloemberg, D. & Quadrilatero, J. Mitophagy regulates mitochondrial network signaling, oxidative stress, and apoptosis during myoblast differentiation. *Autophagy* **15**, 1606–1619 (2019).
117. Gautier, C. A. et al. Regulation of mitochondrial permeability transition pore by PINK1. *Mol. Neurodegener.* **7**, 22 (2012).
118. Sun, T. et al. Parkin regulates programmed necrosis and myocardial ischemia/reperfusion injury by targeting cyclophilin-D. *Antioxid. Redox Signal.* **31**, 1177–1193 (2019).
119. Hom, J. R. et al. The permeability transition pore controls cardiac mitochondrial maturation and myocyte differentiation. *Dev. Cell* **21**, 469–478 (2011).
This work provides the first evidence that the mPTP is important for mammalian development, especially in heart formation.
120. Lingan, J. V., Alanzalon, R. E. & Porter, G. A. Jr. Preventing permeability transition pore opening increases mitochondrial maturation, myocyte differentiation and cardiac function in the neonatal mouse heart. *Pediatr. Res.* **81**, 932–941 (2017).
121. Gong, G. et al. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science* **350**, aad2459 (2015).
122. Cho, S. W. et al. Dual modulation of the mitochondrial permeability transition pore and redox signaling synergistically promotes cardiomyocyte differentiation from pluripotent stem cells. *J. Am. Heart Assoc.* **3**, e000693 (2014).
123. Ying, Z. et al. Short-term mitochondrial permeability transition pore opening modulates histone lysine methylation at the early phase of somatic cell reprogramming. *Cell Metab.* **28**, 935–945 e935 (2018).
124. Tiemeier, G. L. et al. Closing the mitochondrial permeability transition pore in hiPSC-derived endothelial cells induces glycocalyx formation and functional maturation. *Stem Cell Rep.* **13**, 803–816 (2019).
125. Ito, K., Bonora, M. & Ito, K. Metabolism as master of hematopoietic stem cell fate. *Int. J. Hematol.* **109**, 18–27 (2019).
126. Folmes, C. D., Ma, H., Mitalipov, S. & Terzic, A. Mitochondria in pluripotent stem cells: stemness regulators and disease targets. *Curr. Opin. Genet. Dev.* **38**, 1–7 (2016).
127. Garcia-Prat, L. et al. Autophagy maintains stemness by preventing senescence. *Nature* **529**, 37–42 (2016).
128. Liu, X. et al. Cyclophilin D deficiency protects against the development of mitochondrial ROS and cellular inflammation in aorta. *Biochem. Biophys. Res. Commun.* **508**, 1202–1208 (2019).

129. Priber, J. et al. Cyclophilin D disruption attenuates lipopolysaccharide-induced inflammatory response in primary mouse macrophages. *Biochem. Cell Biol.* **93**, 241–250 (2015).
130. Veres, B. et al. Cyclophilin D-dependent mitochondrial permeability transition amplifies inflammatory reprogramming in endotoxemia. *FEBS Open Bio* **11**, 684–704 (2021).
131. Dubinin, M. V. et al. Alisporivir improves mitochondrial function in skeletal muscle of mdx mice but suppresses mitochondrial dynamics and biogenesis. *Int. J. Mol. Sci.* **22**, 9780 (2021).
132. Gao, J. et al. Mitochondrial permeability transition pore in inflammatory apoptosis of human conjunctival epithelial cells and T cells: effect of cyclosporin A. *Invest. Ophthalmol. Vis. Sci.* **54**, 4717–4733 (2013).
133. Alvarez, V. M., Rama Rao, K. V., Brahmbhatt, M. & Norenberg, M. D. Interaction between cytokines and ammonia in the mitochondrial permeability transition in cultured astrocytes. *J. Neurosci. Res.* **89**, 2028–2040 (2011).
134. Roca, F. J., Whitworth, L. J., Redmond, S., Jones, A. A. & Ramakrishnan, L. TNF induces pathogenic programmed macrophage necrosis in tuberculosis through a mitochondrial-lysosomal-endoplasmic reticulum circuit. *Cell* **178**, 1344–1361 e1311 (2019).
135. Garcia, N. & Chavez, E. Mitochondrial DNA fragments released through the permeability transition pore correspond to specific gene size. *Life Sci.* **81**, 1160–1166 (2007).
136. Patrushev, M. et al. Release of mitochondrial DNA fragments from brain mitochondria of irradiated mice. *Mitochondrion* **6**, 43–47 (2006).
137. Nakahira, K. et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* **12**, 222–230 (2011).
138. Allam, R. et al. Mitochondrial apoptosis is dispensable for NLRP3 inflammasome activation but non-apoptotic caspase-8 is required for inflammasome priming. *EMBO Rep.* **15**, 982–990 (2014).
139. Zamudio, F. et al. TDP-43 mediated blood-brain barrier permeability and leukocyte infiltration promote neurodegeneration in a low-grade systemic inflammation mouse model. *J. Neuroinflammation* **17**, 283 (2020).
140. Spiller, K. J. et al. Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 proteinopathy. *Nat. Neurosci.* **21**, 329–340 (2018).
141. Leal-Lasarte, M. M., Franco, J. M., Labrador-Garrido, A., Pozo, D. & Roodveldt, C. Extracellular TDP-43 aggregates target MAPK/MAK/MRK overlapping kinase (MOK) and trigger caspase-3/IL-18 signaling in microglia. *FASEB J.* **31**, 2797–2816 (2017).
142. Yu, C. H. et al. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. *Cell* **183**, 636–649.e18 (2020).
This study describes the participation of the mPTP in the release of mtDNA as an initiator of inflammation.
143. Hausenloy, D. J. et al. Mitochondrial ion channels as targets for cardioprotection. *J. Cell Mol. Med.* **24**, 7102–7114 (2020).
144. Halestrap, A. P. & Richardson, A. P. The mitochondrial permeability transition: a current perspective on its identity and role in ischaemia/reperfusion injury. *J. Mol. Cell Cardiol.* **78**, 129–141 (2015).
145. Ong, S. G. et al. HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. *Cardiovasc. Res.* **104**, 24–36 (2014).
146. Cheung, E. C., Ludwig, R. L. & Vousden, K. H. Mitochondrial localization of TIGAR under hypoxia stimulates HK2 and lowers ROS and cell death. *Proc. Natl Acad. Sci. USA* **109**, 20491–20496 (2012).
147. Liu, R. R. & Murphy, T. H. Reversible cyclosporin A-sensitive mitochondrial depolarization occurs within minutes of stroke onset in mouse somatosensory cortex in vivo: a two-photon imaging study. *J. Biol. Chem.* **284**, 36109–36117 (2009).
148. Adachi, M., Takahashi, K., Nishikawa, M., Miki, H. & Uyama, M. High intraocular pressure-induced ischemia and reperfusion injury in the optic nerve and retina in rats. *Graefes Arch. Clin. Exp. Ophthalmol.* **234**, 445–451 (1996).
149. Hwang, J. H. et al. Cyclosporine A attenuates hypoxic-ischemic brain injury in newborn rats. *Brain Res.* **1359**, 208–215 (2010).
150. Granger, D. N. & Kvietys, P. R. Reperfusion injury and reactive oxygen species: the evolution of a concept. *Redox Biol.* **6**, 524–551 (2015).
151. Crompton, M. The mitochondrial permeability transition pore and its role in cell death. *Biochem. J.* **341**, 233–249 (1999).
152. Griffiths, E. J. & Halestrap, A. P. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem. J.* **307**, 93–98 (1995).
This work provides the first report of the pathological manifestation of the mPTP: cardiac reperfusion injury.
153. Morciano, G. et al. Mechanistic role of mPTP in ischemia-reperfusion injury. *Adv. Exp. Med. Biol.* **982**, 169–189 (2017).
154. Upadhaya, S. et al. Impact of cyclosporine A use in the prevention of reperfusion injury in acute myocardial infarction: a meta-analysis. *Cardiol. J.* **24**, 43–50 (2017).
155. Pottecher, J. et al. Skeletal muscle ischemia-reperfusion injury and cyclosporine A in the aging rat. *Fundam. Clin. Pharmacol.* **30**, 216–225 (2016).
156. Uchino, H. et al. Differential neuroprotection by cyclosporin A and FK506 following ischemia corresponds with differing abilities to inhibit calcineurin and the mitochondrial permeability transition. *Neurobiol. Dis.* **10**, 219–233 (2002).
157. Konukoglu, D., Tasci, I. & Cetinkale, O. Effects of cyclosporin A and ibuprofen on liver ischemia-reperfusion injury in the rat. *Clin. Chim. Acta* **275**, 1–8 (1998).
158. Li, J., Yan, Z. & Fang, Q. A mechanism study underlying the protective effects of cyclosporine-a on lung ischemia-reperfusion injury. *Pharmacology* **100**, 83–90 (2017).
159. Yazdani, I., Majdani, R., Ghasemnejad-Berenji, M. & Dehpour, A. R. Comparison of multiple doses of cyclosporine A on germ cell apoptosis and epididymal sperm parameters after testicular ischemia/reperfusion in rats. *Exp. Mol. Pathol.* **110**, 104271 (2019).
160. Ruiz-Meana, M. et al. The role of mitochondrial permeability transition in reperfusion-induced cardiomyocyte death depends on the duration of ischemia. *Basic. Res. Cardiol.* **106**, 1259–1268 (2011).
161. Imahashi, K. et al. Cardiac-specific ablation of the Na^{+}-Ca^{2+} exchanger confers protection against ischemia/reperfusion injury. *Circ. Res.* **97**, 916–921 (2005).
162. Imahashi, K., Schneider, M. D., Steenbergen, C. & Murphy, E. Transgenic expression of Bcl-2 modulates energy metabolism, prevents cytosolic acidification during ischemia, and reduces ischemia/reperfusion injury. *Circ. Res.* **95**, 734–741 (2004).
163. Luongo, T. S. et al. The mitochondrial Na^{+}/Ca^{2+} exchanger is essential for Ca2+homeostasis and viability. *Nature* **545**, 93–97 (2017).
164. Zhao, Q. et al. The role of the mitochondrial calcium uniporter in cerebral ischemia/reperfusion injury in rats involves regulation of mitochondrial energy metabolism. *Mol. Med. Rep.* **7**, 1073–1080 (2013).
165. Luongo, T. S. et al. The mitochondrial calcium uniporter matches energetic supply with cardiac workload during stress and modulates permeability transition. *Cell Rep.* **12**, 23–34 (2015).
166. Mammucari, C., Raffaello, A., Vecellio Reane, D. & Rizzuto, R. Molecular structure and pathophysiological roles of the mitochondrial calcium uniporter. *Biochim. Biophys. Acta* **1863**, 2457–2464 (2016).
167. Morciano, G. et al. A naturally occurring mutation in ATP synthase subunit c is associated with increased damage following hypoxia/reoxygenation in STEMI patients. *Cell Rep.* **35**, 108983 (2021).
This study identifies for the first time a mutation in a molecular component of the mPTP associated with negative outcome of heart infarction.
168. Cardenas, M. E., Zhu, D. & Heitman, J. Molecular mechanisms of immunosuppression by cyclosporine, FK506, and rapamycin. *Curr. Opin. Nephrol. Hypertens.* **4**, 472–477 (1995).
169. El Baradie, K. B. Y. et al. The cyclophilin inhibitor NIM-811 increases muscle cell survival with hypoxia in vitro and improves gait performance following ischemia-reperfusion in vivo. *Sci. Rep.* **11**, 6152 (2021).
170. Gomez, L. et al. Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice. *Am. J. Physiol. Heart Circ. Physiol.* **293**, H1654–H1661 (2007).
171. Panel, M. et al. Small-molecule inhibitors of cyclophilins block opening of the mitochondrial permeability transition pore and protect mice from hepatic ischemia/reperfusion injury. *Gastroenterology* **157**, 1368–1382 (2019).
172. Fancelli, D. et al. Cinnamic anilides as new mitochondrial permeability transition pore inhibitors endowed with ischemia-reperfusion injury protective effect in vivo. *J. Med. Chem.* **57**, 5333–5347 (2014).
173. Antonucci, S. et al. A novel class of cardioprotective small-molecule PTP inhibitors. *Pharmacol. Res.* **151**, 104548 (2020).
174. Morciano, G. et al. Discovery of novel 1,3,8-triazaspiro[4.5]decane derivatives that target the c subunit of F1/FO-adenosine triphosphate (ATP) synthase for the treatment of reperfusion damage in myocardial infarction. *J. Med. Chem.* **61**, 7131–7143 (2018).
175. Botker, H. E., Cabrera-Fuentes, H. A., Ruiz-Meana, M., Heusch, G. & Ovize, M. Translational issues for mitoprotective agents as adjunct to reperfusion therapy in patients with ST-segment elevation myocardial infarction. *J. Cell Mol. Med.* **24**, 2717–2729 (2020).
176. Linkermann, A., De Zen, F., Weinberg, J., Kunzendorf, U. & Krautwald, S. Programmed necrosis in acute kidney injury. *Nephrol. Dial. Transpl.* **27**, 3412–3419 (2012).
177. Mulay, S. R. et al. Mitochondria permeability transition versus necroptosis in oxalate-induced AKI. *JASN* **30**, 1857–1869 (2019).
178. Ishimoto, Y. & Inagi, R. Mitochondria: a therapeutic target in acute kidney injury. *Nephrol. Dial. Transpl.* **31**, 1062–1069 (2016).
179. Bao, H. et al. Inhibition of glycogen synthase kinase-3beta prevents NSAID-induced acute kidney injury. *Kidney Int.* **81**, 662–673 (2012).
180. Wang, Z. et al. Redox-sensitive glycogen synthase kinase 3beta-directed control of mitochondrial permeability transition: rheostatic regulation of acute kidney injury. *Free Radic. Biol. Med.* **65**, 849–858 (2013).
181. Linkermann, A. et al. Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proc. Natl Acad. Sci. USA* **110**, 12024–12029 (2013).
182. Beck, S. J. et al. Deregulation of mitochondrial F1FO-ATP synthase via OSCP in Alzheimer's disease. *Nat. Commun.* **7**, 11483 (2016).
183. Du, H. et al. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat. Med.* **14**, 1097–1105 (2008).
184. Gomez-Lazaro, M. et al. 6-Hydroxydopamine activates the mitochondrial apoptosis pathway through p38 MAPK-mediated, p53-independent activation of Bax and PUMA. *J. Neurochem.* **104**, 1599–1612 (2008).
185. Martin, L. J., Semenkow, S., Hanaford, A. & Wong, M. Mitochondrial permeability transition pore regulates Parkinson's disease development in mutant alpha-synuclein transgenic mice. *Neurobiol. Aging* **35**, 1132–1152 (2014).
186. Sun, A. et al. Mitochondrial aldehyde dehydrogenase 2 plays protective roles in heart failure after myocardial infarction via suppression of the cytosolic JNK/p53 pathway in mice. *J. Am. Heart Assoc.* **3**, e000779 (2014).
187. Manczak, M. & Reddy, P. H. Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. *Hum. Mol. Genet.* **21**, 5131–5146 (2012).
188. Devi, L., Raghavendran, V., Prabhu, B. M., Avadhani, N. G. & Anandatheerthavarada, H. K. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. *J. Biol. Chem.* **283**, 9089–9100 (2008).
189. Yao, J. et al. Inhibition of amyloid-beta (Abeta) peptide-binding alcohol dehydrogenase-Abeta interaction reduces Abeta accumulation and improves mitochondrial function in a mouse model of Alzheimer's disease. *J. Neurosci.* **31**, 2313–2320 (2011).
190. Liu, G. et al. α-Synuclein is differentially expressed in mitochondria from different rat brain regions and dose-dependently down-regulates complex I activity. *Neurosci. Lett.* **454**, 187–192 (2009).
191. Zhu, Y. et al. α-Synuclein overexpression impairs mitochondrial function by associating with adenylate translocator. *Int. J. Biochem. Cell Biol.* **43**, 732–741 (2011).
192. Sileikyte, J. & Forte, M. The mitochondrial permeability transition in mitochondrial disorders. *Oxid. Med. Cell Longev.* **2019**, 3403075 (2019).
193. Dagda, R. K., Das Banerjee, T. & Janda, E. How Parkinsonian toxins dysregulate the autophagy machinery. *Int. J. Mol. Sci.* **14**, 22163–22189 (2013).

194. Rasheed, M. Z., Tabassum, H. & Parvez, S. Mitochondrial permeability transition pore: a promising target for the treatment of Parkinson's disease. *Protoplasma* **254**, 33–42 (2017).
195. Tillement, L., Lecanu, L., Yao, W., Greeson, J. & Papadopoulos, V. The spirostenol (22R, 25R)-20alpha-spirost-5-en-3beta-yl hexanoate blocks mitochondrial uptake of Abeta in neuronal cells and prevents Abeta-induced impairment of mitochondrial function. *Steroids* **71**, 725–735 (2006).
196. Xie, H. et al. Mitochondrial alterations near amyloid plaques in an Alzheimer's disease mouse model. *J. Neurosci.* **33**, 17042–17051 (2013).
197. Atlante, A. et al. A peptide containing residues 26–44 of tau protein impairs mitochondrial oxidative phosphorylation acting at the level of the adenine nucleotide translocator. *Biochim. Biophys. Acta* **1777**, 1289–1300 (2008).
198. Ludtmann, M. H. R. et al. alpha-synuclein oligomers interact with ATP synthase and open the permeability transition pore in Parkinson's disease. *Nat. Commun.* **9**, 2293 (2018).
199. Du, F. et al. PINK1 signalling rescues amyloid pathology and mitochondrial dysfunction in Alzheimer's disease. *Brain* **140**, 3233–3251 (2017).
200. Fang, E. F. et al. Mitophagy inhibits amyloid-beta and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. *Nat. Neurosci.* **22**, 401–412 (2019).
201. Haroon, M. F. et al. Minocycline, a possible neuroprotective agent in Leber's hereditary optic neuropathy (LHON): studies of cybrid cells bearing 11,778 mutation. *Neurobiol. Dis.* **28**, 237–250 (2007).
202. Cotan, D. et al. Secondary coenzyme Q10 deficiency triggers mitochondria degradation by mitophagy in MELAS fibroblasts. *FASEB J.* **25**, 2669–2687 (2011).
203. Wong, A. & Cortopassi, G. mtDNA mutations confer cellular sensitivity to oxidant stress that is partially rescued by calcium depletion and cyclosporin A. *Biochem. Biophys. Res. Commun.* **239**, 139–145 (1997).
204. Burelle, Y. et al. Mitochondrial vulnerability and increased susceptibility to nutrient-induced cytotoxicity in fibroblasts from leigh syndrome French Canadian patients. *PLoS ONE* **10**, e0120767 (2015).
205. Martin, L. J. et al. GNX-4728, a novel small molecule drug inhibitor of mitochondrial permeability transition, is therapeutic in a mouse model of amyotrophic lateral sclerosis. *Front. Cell Neurosci.* **8**, 433 (2014).
206. Valasani, K. R. et al. Identification of a small molecule cyclophilin D inhibitor for rescuing Aβ-mediated mitochondrial dysfunction. *ACS Med. Chem. Lett.* **7**, 294–299 (2016).
207. Parone, P. A. et al. Enhancing mitochondrial calcium buffering capacity reduces aggregation of misfolded SOD1 and motor neuron cell death without extending survival in mouse models of inherited amyotrophic lateral sclerosis. *J. Neurosci.* **33**, 4657–4671 (2013).
208. Rottenberg, H. & Wu, S. Mitochondrial dysfunction in lymphocytes from old mice: enhanced activation of the permeability transition. *Biochem. Biophys. Res. Commun.* **240**, 68–74 (1997).
209. Goodell, S. & Cortopassi, G. Analysis of oxygen consumption and mitochondrial permeability with age in mice. *Mech. Ageing Dev.* **101**, 245–256 (1998).
210. Mather, M. & Rottenberg, H. Aging enhances the activation of the permeability transition pore in mitochondria. *Biochem. Biophys. Res. Commun.* **273**, 603–608 (2000).
211. Shen, E. Z. et al. Mitoflash frequency in early adulthood predicts lifespan in Caenorhabditis elegans. *Nature* **508**, 128–132 (2014).
212. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194–1217 (2013).
213. Lautrup, S., Sinclair, D. A., Mattson, M. P. & Fang, E. F. NAD^+ in brain aging and neurodegenerative disorders. *Cell Metab.* **30**, 630–655 (2019).
214. Schriewer, J. M., Peek, C. B., Bass, J. & Schumacker, P. T. ROS-mediated PARP activity undermines mitochondrial function after permeability transition pore opening during myocardial ischemia-reperfusion. *J. Am. Heart Assoc.* **2**, e000159 (2013).
215. Gauba, E., Guo, L. & Du, H. Cyclophilin D promotes brain mitochondrial F1FO ATP synthase dysfunction in aging mice. *J. Alzheimers Dis.* **55**, 1351–1362 (2017).
216. Vereczki, V. et al. Cyclophilin D regulates lifespan and protein expression of aging markers in the brain of mice. *Mitochondrion* **34**, 115–126 (2017).
217. Diot, A., Morten, K. & Poulton, J. Mitophagy plays a central role in mitochondrial ageing. *Mamm. Genome* **27**, 381–395 (2016).
218. Markaki, M., Palikaras, K. & Tavernarakis, N. Novel insights into the anti-aging role of mitophagy. *Int. Rev. Cell Mol. Biol.* **340**, 169–208 (2018).
219. Sun, N., Youle, R. J. & Finkel, T. The mitochondrial basis of aging. *Mol. Cell* **61**, 654–666 (2016).
220. Madeo, F., Zimmermann, A., Maiuri, M. C. & Kroemer, G. Essential role for autophagy in life span extension. *J. Clin. Invest.* **125**, 85–93 (2015).
221. Hansen, M., Rubinsztein, D. C. & Walker, D. W. Autophagy as a promoter of longevity: insights from model organisms. *Nat. Rev. Mol. Cell Biol.* **19**, 579–593 (2018).
222. Fernandez, A. F. et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature* **558**, 136–140 (2018).
223. Aspernig, H. et al. Mitochondrial perturbations couple mTORC2 to autophagy in *C.elegans*. *Cell Rep.* **29**, 1399–1409.e5 (2019).
224. Zhou, B. et al. Mitochondrial permeability uncouples elevated autophagy and lifespan extension. *Cell* **177**, 299–314.e16 (2019).
This study provides evidence that the mPTP can determine the impact of autophagy on lifespan.
225. Aggarwal, V. et al. Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. *Biomolecules* **9**, 735 (2019).
226. Giorgi, C. et al. Mitochondria and reactive oxygen species in aging and age-related diseases. *Int. Rev. Cell Mol. Biol.* **340**, 209–344 (2018).
227. Marchi, S. et al. Akt-mediated phosphorylation of MICU1 regulates mitochondrial Ca^{2+} levels and tumor growth. *EMBO J.* **38**, e99435 (2019).
228. Bittremieux, M., Parys, J. B., Pinton, P. & Bultynck, G. ER functions of oncogenes and tumor suppressors: modulators of intracellular Ca^{2+} signaling. *Biochim. Biophys. Acta* **1863**, 1364–1378 (2016).
229. Rimessi, A., Marchi, S., Patergnani, S. & Pinton, P. H-Ras-driven tumoral maintenance is sustained through caveolin-1-dependent alterations in calcium signaling. *Oncogene* **33**, 2329–2340 (2014).
230. Bononi, A. et al. Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ.* **20**, 1631–1643 (2013).
231. Giorgi, C. et al. p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+}-dependent manner. *Proc. Natl Acad. Sci. USA* **112**, 1779–1784 (2015).
232. Giorgi, C. et al. Intravital imaging reveals p53-dependent cancer cell death induced by phototherapy via calcium signaling. *Oncotarget* **6**, 1435–1445 (2015).
233. Bononi, A. et al. BAP1 regulates IP3R3-mediated Ca^{2+} flux to mitochondria suppressing cell transformation. *Nature* **546**, 549–553 (2017).
234. Giorgi, C. et al. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science* **330**, 1247–1251 (2010).
This work, together with Marchi et al. (2019), Bittremieux et al. (2016), Rimessi et al. (2014), Bononi et al. (2013), both Giorgi et al. (2015) and Bononi et al. (2017), demonstrate roles for oncogenes and oncosuppressor genes in the regulation of mitochondrial Ca^{2+} and the regulation of cell death.
235. Vaseva, A. V. et al. p53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell* **149**, 1536–1548 (2012).
236. Bergeaud, M. et al. Mitochondrial p53 mediates a transcription-independent regulation of cell respiration and interacts with the mitochondrial F_1F_0-ATP synthase. *Cell Cycle* **12**, 2781–2793 (2013).
237. Lebedev, I. et al. A novel in vitro CypD-mediated p53 aggregation assay suggests a model for mitochondrial permeability transition by chaperone systems. *J. Mol. Biol.* **428**, 4154–4167 (2016).
238. Hagn, F. et al. Structural analysis of the interaction between Hsp90 and the tumor suppressor protein p53. *Nat. Struct. Mol. Biol.* **18**, 1086–1093 (2011).
239. Giorgio, V., Fogolari, F., Lippe, G. & Bernardi, P. OSCP subunit of mitochondrial ATP synthase: role in regulation of enzyme function and of its transition to a pore. *Br. J. Pharmacol.* **176**, 4247–4257 (2019).
240. Dolder, M., Walzel, B., Speer, O., Schlattner, U. & Wallimann, T. Inhibition of the mitochondrial permeability transition by creatine kinase substrates. Requirement for microcompartmentation. *J. Biol. Chem.* **278**, 17760–17766 (2003).
241. Chappell, J. B. & Crofts, A. R. Calcium ion accumulation and volume changes of isolated liver mitochondria. calcium ion-induced swelling. *Biochem. J.* **95**, 378–386 (1965).
242. Azzi, A. & Azzone, G. F. Swelling and shrinkage phenomena in liver mitochondria. III. Irreversible swelling induced by inorganic phosphate and Ca^{2+}. *Biochim. Biophys. Acta* **113**, 438–444 (1966).
243. Sambri, I. et al. Impaired flickering of the permeability transition pore causes SPG7 spastic paraplegia. *EBioMedicine* **61**, 103050 (2020).
244. Shore, E. R. et al. Small molecule inhibitors of cyclophilin D to protect mitochondrial function as a potential treatment for acute pancreatitis. *J. Med. Chem.* **59**, 2596–2611 (2016).
245. Irwin, W. A. et al. Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency. *Nat. Genet.* **35**, 367–371 (2003).
246. Davis, T. L. et al. Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. *PLoS Biol.* **8**, e1000439 (2010).
247. Masubuchi, Y., Suda, C. & Horie, T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. *J. Hepatol.* **42**, 110–116 (2005).
248. Hansson, M. J. et al. The nonimmunosuppressive cyclosporin analogs NIM811 and UNIL025 display nanomolar potencies on permeability transition in brain-derived mitochondria. *J. Bioenerg. Biomembr.* **36**, 407–413 (2004).
249. Chen, V. M. et al. Collagen VII deficient mice show morphologic and histologic corneal changes that phenotypically mimic human dystrophic epidermolysis bullosa of the eye. *Exp. Eye Res.* **175**, 133–141 (2018).
250. Tiepolo, T. et al. The cyclophilin inhibitor Debio 025 normalizes mitochondrial function, muscle apoptosis and ultrastructural defects in Col6a1$^{-/-}$ myopathic mice. *Br. J. Pharmacol.* **157**, 1045–1052 (2009).
251. Reutenauer, J., Dorchies, O. M., Patthey-Vuadens, O., Vuagniaux, G. & Ruegg, U. T. Investigation of Debio 025, a cyclophilin inhibitor, in the dystrophic mdx mouse, a model for Duchenne muscular dystrophy. *Br. J. Pharmacol.* **155**, 574–584 (2008).
252. Alavian, K. N. et al. The mitochondrial complex V-associated large-conductance inner membrane current is regulated by cyclosporine and dexpramipexole. *Mol. Pharmacol.* **87**, 1–8 (2015).
253. Kang, B. H. et al. Preclinical characterization of mitochondria-targeted small molecule hsp90 inhibitors, gamitrinibs, in advanced prostate cancer. *Clin. Cancer Res.* **16**, 4779–4788 (2010).
254. Richardson, A. P. & Halestrap, A. P. Quantification of active mitochondrial permeability transition pores using GNX-4975 inhibitor titrations provides insights into molecular identity. *Biochem. J.* **473**, 1129–1140 (2016).
255. Warne, J. et al. Selective inhibition of the mitochondrial permeability transition pore protects against neurodegeneration in experimental multiple sclerosis. *J. Biol. Chem.* **291**, 4356–4373 (2016).
256. Sileikyte, J. etal. in *Probe Reports from the NIH Molecular Libraries Program* (National Center for Biotechnology Information, 2010).
257. Waldmeier, P. C., Feldtrauer, J. J., Qian, T. & Lemasters, J. J. Inhibition of the mitochondrial permeability transition by the nonimmunosuppressive cyclosporin derivative NIM811. *Mol. Pharmacol.* **62**, 22–29 (2002).
258. Shares, B. H. et al. Inhibition of the mitochondrial permeability transition improves bone fracture repair. *Bone* **137**, 115391 (2020).
259. Garbaisz, D. et al. Attenuation of skeletal muscle and renal injury to the lower limb following ischemia-reperfusion using mPTP inhibitor NIM-811. *PLoS ONE* **9**, e101067 (2014).
260. Korde, A. S. et al. Protective effects of NIM811 in transient focal cerebral ischemia suggest involvement of the mitochondrial permeability transition. *J. Neurotrauma* **24**, 895–908 (2007).
261. Clarke, S. J., McStay, G. P. & Halestrap, A. P. Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. *J. Biol. Chem.* **277**, 34793–34799 (2002).
262. Tanaka, T. et al. Glycogen synthase kinase-3beta opens mitochondrial permeability transition pore through mitochondrial hexokinase II dissociation. *J. Physiol. Sci.* **68**, 865–871 (2018).
263. Shteinfer-Kuzmine, A., Amsalem, Z., Arif, T., Zooravlov, A. & Shoshan-Barmatz, V. Selective

induction of cancer cell death by VDAC1-based peptides and their potential use in cancer therapy. *Mol. Oncol.* **12**, 1077–1103 (2018).
264. Miyamoto, S., Murphy, A. N. & Brown, J. H. Akt mediates mitochondrial protection in cardiomyocytes through phosphorylation of mitochondrial hexokinase-II. *Cell Death Differ.* **15**, 521–529 (2008).
265. Sun, J. et al. Desensitizing mitochondrial permeability transition by ERK-cyclophilin D axis contributes to the neuroprotective effect of gallic acid against cerebral ischemia/reperfusion injury. *Front. Pharmacol.* **8**, 184 (2017).
266. Wu, R. et al. Hexokinase II knockdown results in exaggerated cardiac hypertrophy via increased ROS production. *EMBO Mol. Med.* **4**, 633–646 (2012).
267. Pravdic, D. et al. Anesthetic-induced preconditioning delays opening of mitochondrial permeability transition pore via protein kinase C-epsilon-mediated pathway. *Anesthesiology* **111**, 267–274 (2009).
268. Shanmughapriya, S. et al. SPG7 Is an essential and conserved component of the mitochondrial permeability transition pore. *Mol. Cell* **60**, 47–62 (2015).
269. Hurst, S., Baggett, A., Csordas, G. & Sheu, S. S. SPG7 targets the m-AAA protease complex to process MCU for uniporter assembly, Ca^{2+} influx, and regulation of mitochondrial permeability transition pore opening. *J. Biol. Chem.* **294**, 10807–10818 (2019).
270. Valasani, K. R., Vangavaragu, J. R., Day, V. W. & Yan, S. S. Structure based design, synthesis, pharmacophore modeling, virtual screening, and molecular docking studies for identification of novel cyclophilin D inhibitors. *J. Chem. Inf. Model.* **54**, 902–912 (2014).
271. Ni, S. et al. Discovering potent small molecule inhibitors of cyclophilin A using de novo drug design approach. *J. Med. Chem.* **52**, 5295–5298 (2009).
272. Guichou, J. F. et al. Structure-based design, synthesis, and biological evaluation of novel inhibitors of human cyclophilin A. *J. Med. Chem.* **49**, 900–910 (2006).
273. Schinzel, A. C. et al. Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc. Natl Acad. Sci. USA* **102**, 12005–12010 (2005).
274. Ades, L., Itzykson, R. & Fenaux, P. Myelodysplastic syndromes. *Lancet* **383**, 2239–2252 (2014).
275. Yu, W. et al. Cyclosporine a suppressed glucose oxidase induced p53 mitochondrial translocation and hepatic cell apoptosis through blocking mitochondrial permeability transition. *Int. J. Biol. Sci.* **12**, 198–209 (2016).
276. Baines, C. P., Kaiser, R. A., Sheiko, T., Craigen, W. J. & Molkentin, J. D. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat. Cell Biol.* **9**, 550–555 (2007).
277. McEnery, M. W., Snowman, A. M., Trifiletti, R. R. & Snyder, S. H. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc. Natl Acad. Sci. USA* **89**, 3170–3174 (1992).
278. Marzo, I. et al. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* **281**, 2027–2031 (1998).
279. Cheng, E. H., Sheiko, T. V., Fisher, J. K., Craigen, W. J. & Korsmeyer, S. J. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science* **301**, 513–517 (2003).
280. Kinnally, K. W. et al. Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. *Proc. Natl Acad. Sci. USA* **90**, 1374–1378 (1993).
281. Beutner, G., Ruck, A., Riede, B. & Brdiczka, D. Complexes between porin, hexokinase, mitochondrial creatine kinase and adenylate translocator display properties of the permeability transition pore. Implication for regulation of permeability transition by the kinases. *Biochim. Biophys. Acta* **1368**, 7–18 (1998).
282. Ruck, A., Dolder, M., Wallimann, T. & Brdiczka, D. Reconstituted adenine nucleotide translocase forms a channel for small molecules comparable to the mitochondrial permeability transition pore. *FEBS Lett.* **426**, 97–101 (1998).
283. Sileikyte, J. et al. Regulation of the mitochondrial permeability transition pore by the outer membrane does not involve the peripheral benzodiazepine receptor (translocator protein of 18kDa (TSPO)). *J. Biol. Chem.* **289**, 13769–13781 (2014).
284. Herick, K., Kramer, R. & Luhring, H. Patch clamp investigation into the phosphate carrier from Saccharomyces cerevisiae mitochondria. *Biochim. Biophys. Acta* **1321**, 207–220 (1997).
285. Leung, A. W., Varanyuwatana, P. & Halestrap, A. P. The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition. *J. Biol. Chem.* **283**, 26312–26323 (2008).
286. Gutierrez-Aguilar, M. et al. Genetic manipulation of the cardiac mitochondrial phosphate carrier does not affect permeability transition. *J. Mol. Cell Cardiol.* **72**, 316–325 (2014).
287. Klutho, P. J., Dashek, R. J., Song, L. & Baines, C. P. Genetic manipulation of SPG7 or NipSnap2 does not affect mitochondrial permeability transition. *Cell Death Discov.* **6**, 5 (2020).
288. Porter, G. A. Jr. & Beutner, G. Cyclophilin D, somehow a master regulator of mitochondrial function. *Biomolecules* **8**, 176 (2018).

Acknowledgements
The Signal Transduction Laboratory is supported by the Italian Association for Cancer Research (IG-23670 to P.P. and IG-19803 to C.G.), A-ROSE, Progetti di Rilevante Interesse Nazionale (PRIN2017E5L5P3 to P.P and PRIN20177E9EPY to C.G.), the Italian Ministry of Health (GR-2013-02356747 to C.G.), the European Research Council (853057-InflaPML to C.G.) and local funds from the University of Ferrara to M.B., C.G. and P.P. P.P. is grateful to C. degli Scrovegni for her continuous support.

Author contributions
All authors researched data for the article, contributed to discussion of the content, wrote the article and edited the manuscript.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Molecular Cell Biology thanks Valentina Giorgio and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41580-021-00433-y.