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REVIEW

Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition

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M Bonora[1], MR Wieckowski[2], C Chinopoulos[3], O Kepp[4,5,6], G Kroemer[4,5,6,7], L Galluzzi[4,5,8,9] and P Pinton[1,9]

The term mitochondrial permeability transition (MPT) is commonly used to indicate an abrupt increase in the permeability of the inner mitochondrial membrane to low molecular weight solutes. Widespread MPT has catastrophic consequences for the cell, *de facto* marking the boundary between cellular life and death. MPT results indeed in the structural and functional collapse of mitochondria, an event that commits cells to suicide via regulated necrosis or apoptosis. MPT has a central role in the etiology of both acute and chronic diseases characterized by the loss of post-mitotic cells. Moreover, cancer cells are often relatively insensitive to the induction of MPT, underlying their increased resistance to potentially lethal cues. Thus, intense efforts have been dedicated not only at the understanding of MPT in mechanistic terms, but also at the development of pharmacological MPT modulators. In this setting, multiple mitochondrial and extramitochondrial proteins have been suspected to critically regulate the MPT. So far, however, only peptidylprolyl isomerase F (best known as cyclophilin D) appears to constitute a key component of the so-called permeability transition pore complex (PTPC), the supramolecular entity that is believed to mediate MPT. Here, after reviewing the structural and functional features of the PTPC, we summarize recent findings suggesting that another of its core components is represented by the c subunit of mitochondrial ATP synthase.

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MITOCHONDRIAL PERMEABILITY TRANSITION AND CELL DEATH

The expression 'mitochondrial permeability transition' (MPT) is commonly used to indicate a brisk increase in the permeability of the inner mitochondrial membrane to low molecular weight solutes (<1.5 kDa). This results in the osmotic influx of water into the mitochondrial matrix, followed by the structural and functional collapse of affected mitochondria.[1,2] According to current models, MPT would be mediated by the so-called permeability transition pore complex (PTPC), a supramolecular entity assembled at the interface between the inner and the outer mitochondrial membranes.[1,3] The first description of MPT dates back to 1979, when this phenomenon was shown to stem from the accumulation of Ca^{2+} ions in the mitochondrial matrix and to be responsive to Mg^{2+} ions as well as ADP.[4] However, the interest in MPT dropped immediately thereafter, as the process could not be given any pathophysiological relevance. It was only in the mid-1990s when it became evident that mitochondria have a central role in the regulation of cell death elicited by several stimuli.[5,6] Indeed, while MPT affecting a limited fraction of mitochondria can be managed by their autophagic removal,[7] widespread MPT commits the cell to death via regulated necrosis or apoptosis (Figure 1).[2] MPT-driven regulated necrosis mainly (but not only) reflects the bioenergetic outcomes of MPT, that is, the immediate dissipation of the mitochondrial transmembrane potential ($\Delta\psi_m$) and the consequent arrest in all $\Delta\psi_m$-dependent mitochondrial activities, including ATP synthesis.[8,9] Conversely, MPT-driven apoptosis is mainly executed by mitochondrial intermembrane proteins that are released into the cytoplasm upon MPT, including (but not limited to) cytochrome *c*, apoptosis-inducing factor, mitochondrion-associated, 1 (AIFM1, best known as AIF) and diablo, IAP-binding mitochondrial protein (DIABLO, also known as Smac).[10–12] As the apoptotic phenotype requires the activation of caspases,[13] a family of cysteine proteases that operate in an ATP-dependent manner,[14] MPT may drive apoptosis or regulated necrosis depending on the intracellular availability of ATP.[15] However, other parameters may determine, at least in part, the catabolic pathways activated by MTP, including the nitrosylation state of caspases,[16] and the expression levels of endogenous caspase modulators.[17–19]

Throughout the last two decades, robust genetic evidence has incriminated MPT as a major etiological determinant in a wide panel of acute and chronic disorders characterized by the unwarranted loss of post-mitotic cells. These conditions include, but are not limited to: (1) ischemia/reperfusion injury of the brain,[20] heart[21–23] and kidney;[24] (2) neurodegenerative disorders;[25] (3) toxic syndromes;[26–28] (4) diabetes;[29] and (5) myopathic/

[1]Section of Pathology, Oncology and Experimental Biology, Laboratory for Technologies of Advanced Therapies (LTTA), Department of Morphology, Surgery and Experimental Medicine, Interdisciplinary Centre for the Study of Inflammation (ICSI), University of Ferrara, Ferrara, Italy; [2]Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; [3]Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary; [4]Equipe 11 labelisée par la Ligue Nationale contre le cancer, INSERM U1138, Centre de Recherche des Cordeliers, Paris, France; [5]Université Paris Descartes/Paris 5, Sorbonne Paris Cité, Paris, France; [6]Metabolomics and Cell Biology platforms, Gustave Roussy Comprehensive Cancer Center, Villejuif, France; [7]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France and [8]Gustave Roussy Comprehensive Cancer Center, Villejuif, France. Correspondence: Dr L Galluzzi or Professor P Pinton, Equipe 11 labelisée par la Ligue Nationale contre le cancer, Centre de Recherche des Cordeliers, 15 Rue de l'ecole de Medecine, F-75006, Paris, France or Section of Pathology, Oncology and Experimental Biology, University of Ferrara, via Fossato di Mortara 70, I-44121, Ferrara, Italy.
E-mail: deadoc@vodafone.it or pnp@unife.it
[9]These authors share senior co-authorship.
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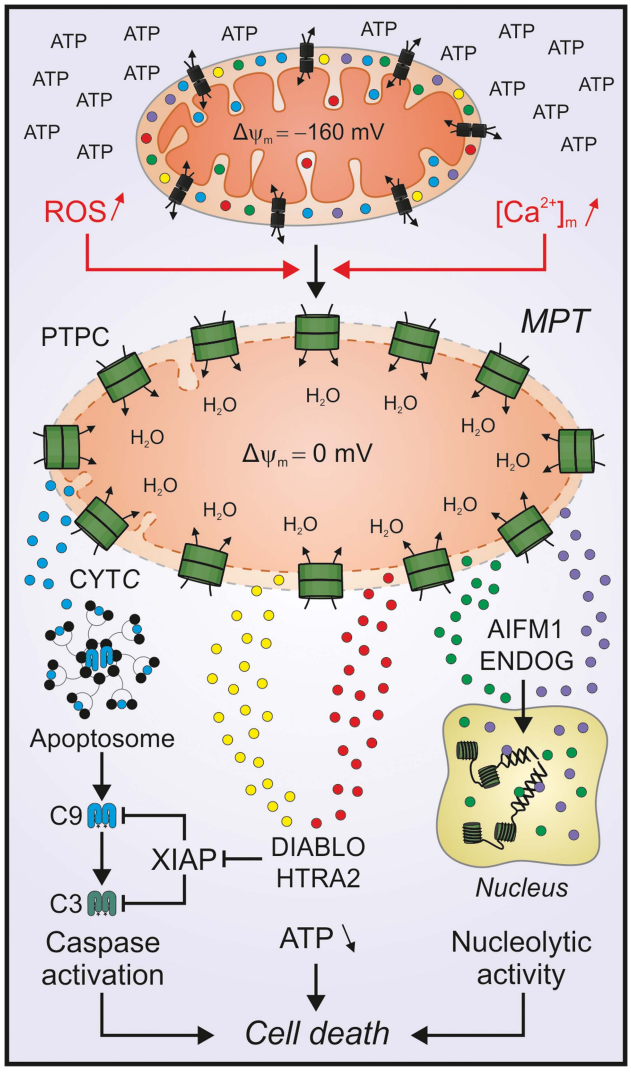

Figure 1. Lethal effects of MPT. When the inner mitochondrial membrane becomes permeable to low molecular weight solutes, positively charged ions massively flow into the mitochondrial matrix driven by its electronegative nature. This phenomenon, which is commonly referred to as MPT, has two major consequences. First, it coincides with the dissipation of the $\Delta\psi_m$, virtually abolishing mitochondrial ATP synthesis and several other $\Delta\psi_m$-dependent mitochondrial functions. Second, it drives the massive entry of water into the mitochondrial matrix, causing an osmotic imbalance that results in the breakdown of both mitochondrial membranes. In turn, this provokes the release into the cytosol of several factors that are normally confined within the intermembrane space, including (but not limited to) cytochrome *c* (CYTC), AIFM1, endonuclease G (ENDOG), DIABLO and HtrA serine peptidase 2 (HTRA2). Thus, depending on multiple parameters, including the global availability of ATP and perhaps the expression levels of caspase inhibitors such as X-linked inhibitor of apoptosis (XIAP), widespread MPT can induce necrotic as well as apoptotic instances of cell death. The latter are dominated by the CYTC-dependent activation of the caspase-9 (C9) → caspase-3 (C3) cascade, which is indirectly favored by both DIABLO and HTRA2. Conversely, the former originate in large part from the bioenergetic crisis that is provoked by MPT coupled to the caspase-independent endonucleolytic activity of AIFM1 and ENDOG. APAF1, apoptotic peptidase-activating factor 1; $[Ca^{2+}]_m$, mitochondrial Ca^{2+} concentration; ROS, reactive oxygen species.

dystrophic disorders.[30,31] Moreover, malignant cells have been shown to exhibit defects in the PTPC or upstream signal transduction cascades, underlying (at least in part) their intrinsic resistance to both endogenous stress and various therapeutic interventions.[3,32] Along with the recognition that MPT has a critical role in multiple pathophysiological scenarios, great interest gathered around the possibilities that (1) pharmacological inhibitors of MPT or mitochondrial outer membrane permeabilization (MOMP),[11,33,34] the major mechanism underlying intrinsic apoptosis, would mediate therapeutically relevant cytoprotective effects;[35] and (2) pharmacological activators of MPT or MOMP could be used to selectively kill neoplastic cells based on their intrinsically elevated levels of stress.[36,37] This translated into an intense wave of investigation that unveiled multiple mechanistic details about MPT and allowed for the characterization of various pharmacological and endogenous MPT modulators.[3,38] Thus, besides the accumulation of mitochondrial Ca^{2+}, major MPT stimulators include reactive oxygen species, inorganic phosphate, intracellular alkalinization, long chain fatty acids, as well as atractyloside and carboxyatractyloside, both of which inhibit members of the adenine nucleotide translocase (ANT) protein family by locking them in cytoplasmic side open conformation.[3] Conversely, among various molecules, MPT is inhibited by ATP and ADP, NADH and NAD^+, glutamate, as well as by bongkrekic acid, which locks ANT family members in a matrix side open conformation, 5-isothiocyanato-2-[2-(4-isothiocyanato-2-sulfophenyl)ethenyl]benzene-1-sulfonic acid (DIDS), an inhibitor of voltage-dependent anion channel (VDACs), and cyclosporine A (CsA), which targets peptidylprolyl isomerase F (PPIF, best known as cyclophilin D, CYPD).[38]

The MPT-inhibitory potential of CsA has been documented so extensively, *in vitro* and *in vivo*, that this molecule is currently considered as the gold standard means for the confirmation of presumed instances of MPT.[39] Nonetheless, caution should be applied to interpret the effects of CsA, especially those observed *in vivo*, as this chemical is endowed with potent immunosuppressive properties (reflecting its ability to indirectly inhibit calcineurin).[40] Thus, to ascribe with relative certainty a murine phenotype to MPT, it is imperative to evaluate the *in vivo* cytoprotective effects of CsA in *Ppif*$^{-/-}$ animals (see below), and to demonstrate that these two experimental interventions show a null epistatic interaction.

In spite of the intense experimental interest generated by MPT throughout the last two decades, the precise molecular composition of the PTPC remains elusive.[41] After summarizing the main structural and functional features of the PTPC discovered so far, here we discuss recent findings suggesting that one of its core components is represented by the c subunit of mitochondrial ATP synthase. A detailed discussion of the molecular mechanisms that control MOMP goes beyond the scope of this review article, and can be found in Tait and Green,[11] Taylor *et al.*,[14] Chipuk *et al.*[33] and Galluzzi *et al.*[35]

ARCHITECTURE OF THE PTPC

Core components

In the early 1990s, electrophysiological studies based on purified mitoplasts (that is, mitochondria stripped of the outer membrane) demonstrated that MPT corresponds to an significant increase in the conductance of the inner mitochondrial membrane,[42] pointing to the existence of a pore that would be responsible for this transition. Such a 'mitochondrial megachannel' was rapidly found to share several features with MPT, including its sensitivity to Ca^{2+} ions (which operate as activators) as well as to CsA and various divalent cations, including Mg^{2+} (all of which operate as inhibitors).[42,43] Shortly thereafter, the mitochondrial megachannel turned out to exhibit a voltage-dependent behavior, in thus far

resembling VDAC.[44] In support of a critical role for VDAC in MPT, purified VDAC molecules reconstituted in planar bilayers or proteoliposomes were shown to form a dimeric channel that exhibited electrophysiological properties compatible with those of the mitochondrial megachannel.[45] Such an unexpected link between a protein of the outer mitochondrial membrane, VDAC, and a phenomenon that involves the inner mitochondrial membrane (i.e., MPT) casted suspicion on the actual composition of the mitochondrial megachannel, raising the possibility that it would be constituted by several proteins, not just one. Further supporting this hypothesis, a ligand of the peripheral benzodiazepine receptor (which was already known to involve VDAC, ANT and a third component)[46] was found to elicit currents from otherwise electrically silent mitoplasts.[44]

Brdiczka and colleagues confirmed the supramolecular nature of the PTPC in 1996, when they documented (in the rat brain) the presence of a complex comprising VDAC, ANT, hexokinase 1 (HK1) and creatine kinase, mitochondrial 1 (CKMT1) and exhibiting MPT-like electrical activity upon reconstitution in liposomes.[47,48] Based on its interacting partners (including VDAC and ANT)[49] as well as on its pharmacological profile,[50,51] CYPD was soon suspected to have a central role in MPT. In the late 1990s, purified ANT molecules reconstituted in proteoliposomes were found to form an oligomeric channel exhibiting PTPC-like functional properties.[52] Cumulatively, these findings inspired a first PTPC model according to which MPT would be mediated by a supramolecular entity assembled at the interface between the inner and outer mitochondrial membrane by the physical and functional interaction of VDAC, ANT, HK1 and CKMT1. In line with its suborganellar localization (the mitochondrial matrix), CYPD was considered by this model as a regulator of the PTPC, but not as one of its pore-forming subunits.

Robust genetic data generated in the mid-2000s significantly challenged most components of its model. Thus, the simultaneous knockout of the genes coding for two distinct ANT isoforms, that is, *Slc25a4* (encoding Ant1) and *Slc25a5* (encoding Ant2), failed to abolish the ability of murine hepatocytes to succumb to several MPT inducers, including the Ca^{2+} ionophore Br-A23187, in a CsA-inhibitable manner.[53] In line with this notion, mitochondria isolated from *Slc25a4*$^{-/-}$*Slc25a5*$^{-/-}$ hepatocytes retained the ability to undergo MPT *in vitro* upon exposure to a depolarizing agent, yet became irresponsive to atractyloside and ADP.[53] Similarly, the simultaneous genetic inactivation of three distinct VDAC isoforms, namely, Vdac1, Vdac2 and Vdac3, neither altered the propensity of murine fibroblasts to die when challenged with hydrogen peroxide (an MPT inducer), nor did it influence the ability of their mitochondria to undergo MPT in response to Ca^{2+}.[54,55] At odds with these relatively minor effects, the standalone deletion of *Ppif* turned out to mediate major MPT-inhibitory and cytoprotective effects, *in vitro* as well as *in vivo*, in several models of acute ischemic injury.[20–22,56] In particular, the absence of CYPD was shown to markedly increase the amount of Ca^{2+} ions required to trigger MPT and to render this process completely insensitive to CsA.[20,56]

Taken together, these data apparently demonstrate that ANT and VDAC are dispensable for both the execution and the regulation of MPT, while CYPD has a crucial role in the process. This said, a central function for ANT in MPT cannot be formally excluded yet, as at least two additional ANT isoforms turned out to be encoded by the mammalian genome, namely, SLC25A6 (ANT3) and SLC25A31 (ANT4).[57,58] So far, no VDAC isoforms other than VDAC1, VDAC2 and VDAC3 have been identified (source http://www.ncbi.nlm.nih.gov/gene/). Nonetheless, the results of Baines *et al*.[54] were obtained with *Vdac1*$^{-/-}$*Vdac3*$^{-/-}$ cells subjected to the temporary depletion of Vdac2 by small-interfering RNAs,[55] an experimental system that appears somehow less robust than the simultaneous deletion of all VDAC-coding genes (which cannot be achieved as the knockout of *Vdac2* is lethal).[59] Finally, it seems unlikely that CYPD, which is mainly localized within the mitochondrial matrix, would constitute the actual pore-forming component of the PTPC. In line with this notion, CYPD is currently viewed as the major gatekeeper of MPT, regulating the opening of the PTPC but not lining up the pore that physically allows for the entry of low molecular weight solutes into the mitochondrial matrix. This said, the possibility that CYPD may change conformation and become able to form pores in the inner mitochondrial membrane during MPT, similar to what BAX does in the course of MOMP,[60] has not yet been formally excluded.

Inorganic phosphate has been identified very early as an MPT-promoting metabolite,[61] suggesting that the PTPC would possess a specific binding site. In physiological conditions, inorganic phosphate is transported across the inner mitochondrial membrane by members of the SLC protein family, including SLC25A3 (best known as PHC or PiC) and SLC25A24 (also known as APC1).[62] Although PiC imports inorganic phosphate into mitochondrial matrix coupled to either the co-import of H^+ ions or the export of OH^- ions, APC1 mediates this process along with the export of ATP and Mg^{2+} ions.[62] In 2003, APC1 was suggested to be responsible for the MPT-promoting activity of inorganic phosphate via an indirect effect on the mitochondrial pool of ATP and ADP,[63] a notion that has not been confirmed. Rather, it seems that APC1 responds to increases in cytosolic Ca^{2+} levels by operating in reverse mode, thus favoring the mitochondrial uptake of ATP and ADP and inhibiting MPT.[64] In 2006, PiC turned out to be the functional target of viral mitochondria-localized inhibitor of apoptosis, an antiapoptotic protein encoded by cytomegalovirus,[65,66] while in 2008 PiC was shown to bind CYPD and ANT1 in cellula, an interaction that was potentiated by MPT-inducing conditions and inhibited by CsA.[67] Along similar lines, a high-throughput genetic screen unveiled that PiC overexpression promotes mitochondrial dysfunction coupled to apoptotic cell death.[68] Also in this study PiC was found to interact with ANT1 (as well as with VDAC1), especially in the presence of MPT inducers.[68] Moreover, the small-interfering RNA-mediated depletion of PiC exerted cytoprotective effects.[68] Together with previous data indicating that the reconstitution of liposomes with purified PiC molecules results in the formation of relatively unspecific pores,[69] these findings pointed to PiC as to the possible pore-forming unit of the PTPC. This hypothesis is incompatible with recent results indicating that a consistent reduction in PiC levels does not alter the ability of isolated mitochondria to undergo MPT in response to Ca^{2+} ions.[70] Thus, either PiC does not participate into the PTPC in a significant manner, or very small amounts of PiC are sufficient to mediate MPT. As a corollary, this suggests that the cytoprotective effects of PiC depletion[68] may not stem from the modulation of MPT. Although the ability of PiC to influence mitochondrial dynamics may be involved in this process,[71] the exact molecular mechanisms by which PiC promotes cell death under some circumstances remain to be elucidated.

Regulatory components

Several proteins have been shown to regulate the activity of core PTPC units (that is, VDAC, ANT and CYPD). These regulatory components, which encompass cytosolic as well as mitochondrial proteins, appear to interact with the PTPC backbone in a highly dynamic manner.[72]

The translocator protein (18 kDa) (TSPO), a protein of the outer mitochondrial membrane, constitutes the benzodiazepine-binding component of the so-called peripheral benzodiazepine receptor, an oligomeric complex involving VDAC and ANT (see above).[46] The physiological role of TSPO in steroid biosynthesis was described as early as in 1989,[73] and only a few years later circumstantial evidence implicating TSPO in MPT began to accumulate. For the most part, these studies reported the ability of a series of endogenous (for example, protoporphyrin IX)[74] and

exogenous (for example, PK11195, Ro5–4864, diazepam)[75,76] TSPO agonists to elicit MPT in isolated mitochondria. In line with this notion, the incubation of purified mitochondria with a TSPO-blocking antibody reportedly inhibits several manifestations of MPT.[77] This said, the effects of TSPO ligands on cell death exhibit a great degree of variability, ranging from cytoprotective,[78,79] to overtly lethal.[80–82] Such a context dependency may stem from several causes, including (but presumably not limited to) model-intrinsic variables (including the expression levels of TSPO and other benzodiazepine receptors) and the concentration of TSPO-modulatory agents used, possibly linked to off-target effects.[36,83]

Various kinases have been shown to physically and/or functionally interact with core PTPC units (at least in specific tissues, such as the brain), including CKMT1 (which is localized in the mitochondrial intermembrane space), HK1, HK2 as well as glycogen synthase kinase 3β (GSK3β) and protein kinase Cε (PKCε).[72] Some of these kinases, including CKMT1, HK1 and HK2 do not phosphorylate protein substrates, implying that their MPT-modulatory activity originates either from their physical interaction with core PTPC components or from their ability to catalyze metabolic reactions. Besides binding VDAC1 and ANT1,[47,48] CKMT1 phosphorylates creatine to generate phosphocreatine, a reaction that is tightly coupled to oxidative phosphorylation (and hence to the availability of ATP and ADP).[84,85] It remains to be formally demonstrated whether the MPT-modulatory activity of CKMT1 originates from its physical interaction with PTPC components or its catalytic activity. HKs catalyze the rate-limiting step of glycolysis, converting glucose into glucose-6-phosphate in an ATP-dependent manner.[86] Both HK1 and HK2 interact with multiple VDAC isoforms, hence gaining a preferential access to ATP exported from mitochondria.[87] This configuration (that is, the binding of HKs to VDAC) is associated with an optimal flux through glycolysis as well as with major cytoprotective effects.[88] Accordingly, the administration of cell-permeant peptides or chemicals that competitively displace HK2 from VDAC1 has been shown to kill several types of cells upon MPT.[89–92] However, it remains unclear to which extent such a cytotoxic response reflects a direct modulation of the PTPC by HK2 rather than an indirect effect on the availability of antioxidants (cancer cells exploit glycolysis to boost the pentose phosphate pathway, which is critical for the regeneration of NAD(P)H and hence reduced glutathione).[93,94] The fact that the MPT-inducing activity of peptides disrupting the HK2/VDAC1 interaction is inhibited by CsA and bongkrekic acid, as well as by the ablation of *Ppif*, but not by that of *Vdac1* and *Vdac3*,[95] suggests that the PTPC-regulatory function of HKs mainly stems from a metabolic effect. Further supporting this notion, HK1 has recently been found to exert major cytoprotective effects in MPT-unrelated paradigms of death.[96]

Contrarily to CKMT1 and HKs, GSK3β and PKCε exert MPT-modulatory functions that have been linked (at least partially) to their ability to phosphorylate core PTPC components.[97–99] For instance, active GSK3β has been reported to phosphorylate VDAC1, resulting in the MPT-stimulatory displacement of HK2,[97] and VDAC2, promoting the consumption of ATP by ischemic mitochondria (a process that is also expected to promote MPT),[100] while GSK3β phosphorylated on Ser9 (that is, inactive) appears to inhibit the PTPC by physically disrupting the ANT1/CYPD interaction.[101] Recently, the activation of GSK3β has also been linked to the MPT-triggering phosphorylation of CYPD.[102,103] However, formal evidence supporting the notion that GSK3β directly phosphorylates CYPD is lacking.[102] PKCε has been reported to phosphorylate VDAC1, yet this post-translational modification appears to promote, rather than destabilize, HK2 binding.[98] However, as the activation of PKCε by a synthetic peptide has been associated with the inactivating dephosphorylation of GSK3β,[104] it is not clear whether the effect of PKCε on the VDAC1/HK2 interaction *in cellula* actually reflects a direct phosphorylation event or a GSK3β-dependent signaling circuitry. As a matter of fact, the activation of several upstream signal transducers, including AKT1, mammalian target of rapamycin (mTOR), protein kinase A and protein kinase, cGMP-dependent, type I (PRKG1, best known as PKG) reportedly converge on the inactivation of GSK3β, hence mediating MPT-inhibitory effects.[99,105,106] A detailed description of these signaling pathways, which have a significant role in ischemic conditioning and cardio-protection, goes largely beyond the scope of this review.[107]

Of note, the core units of the PTPC have been shown to interact with several components of the machinery that control MOMP, including both pro- and anti-apoptotic members of the Bcl-2 protein family[59,108–116] as well as p53.[117,118] BCL-2 and BCL-2-like 1 (BCL-2L1, best known as BCL-X_L) have been proposed to inhibit MPT by regulating the opening state of VDAC1.[111,112] This said, whether the MPT-modulatory activity of anti-apoptotic BCL-2 family members originates from an increase or a decrease in VDAC1 conductance remains a matter of debate. Irrespective of this uncertainty, BAX, BAK1 and BCL-2-like 11 (BCL-2L11, a BH3-only protein best known as BID) reportedly promote MPT-driven apoptosis by interacting with ANT1 and/or VDAC1.[108,110,119] Along similar lines, BCL-2-associated agonist of cell death (BAD, another BH3-only protein) has been shown to trigger a VDAC1-dependent, BCL-X_L-responsive mechanism of MPT.[113] In this context, however, MPT appears to result from the BAD-dependent displacement of BCL-X_L from VDAC1 rather than from a physical BAD/VDAC1 interaction.[113] Finally, by sequestering the BAX-like protein BAK1, VDAC2 reportedly exerts MOMP-inhibitory functions.[59] Thus, the molecular machineries for MOMP and MPT engage in complex, mutually regulatory crosstalk.

Recent data indicate that a pool of p53 localized to the mitochondrial matrix participate in MPT-driven regulated necrosis by interacting with CYPD.[117] These findings add to an increasing amount of data arguing against the classical apoptosis/necrosis dichotomy. BAX and BAK1 are indeed being implicated in several paradigms of necrotic, as opposed to apoptotic, cell death,[23,120] perhaps reflecting their ability to regulate mitochondrial dynamics,[23] or Ca^{2+} homeostasis.[121–126] Further studies are required to obtain precise insights into this issue.

In summary, in spite of a significant experimental effort, the precise molecular composition of the PTPC remains elusive (Figure 2). Accumulating evidence indicate that the mitochondrial ATP synthase, the multiprotein complex that catalyzes the synthesis of ATP while dissipating the chemiosmotic gradient generated by the respiratory chain across the inner mitochondrial membrane, constitutes a central PTPC component, as discussed below.

MITOCHONDRIAL ATP SYNTHASE: STRUCTURE, FUNCTION AND IMPLICATION IN MPT

Molecular composition of mitochondrial ATP synthase

The mitochondrial ATP synthase is a large multiprotein complex consisting of a globular domain that protrudes into the mitochondrial matrix (F_1 domain, also known as soluble component) and an inner mitochondrial membrane-embedded domain (F_O domain), which are interconnected by a central and a lateral stalk. Owing to this molecular arrangement, the ATP synthase is also known as F_1F_O-ATPase.[127] Mammalian ATP synthases contain 15 different subunits: α, β, γ, δ, ε, a, b, c, d, e, f, g, A6L, F6 and O (also known as oligomycin sensitivity-conferring protein, OSCP) forming a fully functional holoenzyme with a total molecular weight of ~600 kDa. The α, β, γ, a and c subunits exhibit a high degree of homology to their chloroplast and bacterial counterparts. Moreover, the overall topology of the mammalian ATP synthase as well as that of its F_1 and F_O components taken individually are highly conserved across evolution.[127–129] The mammalian F_1 domain is composed of three α/β dimers and interacts with one copy of the γ, δ and ε subunits (central stalk) as well as with the b, d, F6 and O subunits (peripheral stalk),

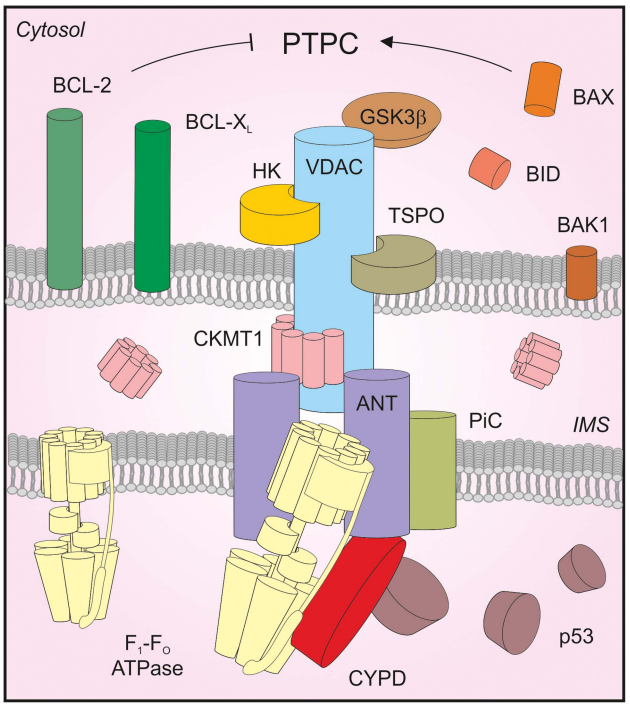

Figure 2. Possible configuration of the PTPC. According to current models, MPT is mediated by the opening of a supramolecular entity assembled at the juxtaposition between mitochondrial membranes. Such a large multiprotein complex is commonly known as PTPC. Structural and functional studies performed throughout the past two decades suggest that multiple mitochondrial and cytosolic proteins intervene in the formation or regulation of the PTPC, yet the actual pore-forming unit of the complex remains elusive. These proteins include (but are not limited to): various isoforms of VDAC, ANT and HK, CYPD, PiC, TSPO, CKMT1, GSK3β, p53, as well as several members of the Bcl-2 protein family. The precise composition of the PTPC, however, remains elusive. Recent data indicate that the mitochondrial ATP synthase, in particular, the c subunit of the F_O domain, has a critical role in MPT. Whether the c subunit truly constitutes the pore-forming unit of the PTPC, however, has not yet been formally demonstrated. IMS, mitochondrial intermembrane space.

providing a physical bridge between the soluble and proton-translocating (F_O) components of the holoenzyme.[129–131] The F_O domain contains a ring-shaped oligomer of c subunits stabilized by binding of cardiolipin, a lipid that is highly enriched in (if not confined to) the inner mitochondrial membrane.[129,132] Of note, the number of c subunits composing the so-called c-ring varies to a significant extent across species (10 in humans).[129] These components of the F_O domain are highly hydrophobic and contain a critical carboxyl group (most often as part of a Glu or Asp residue) that is directly involved in the translocation of H^+ ions across the inner mitochondrial membrane (see below).[133] The remaining constituents of ATP synthase, that is, the a, e, f, g and A6L subunits, are also part of the F_O domain and interact with the c-ring. In particular, the a subunit provides a physical dock for the b subunit, while A6L appears to bridge F_O to other components of the peripheral stalk (Figure 3).[129–131,134]

The roles of individual F_1F_O-ATPase subunits in ATP synthesis

Mitchell's chemiosmotic model, which is still largely accepted, postulated that the F_1F_O-ATPase is able to dissipate in a controlled manner the electrochemical gradient generated across the inner mitochondrial membrane by respiratory chain complexes to condense ADP and inorganic phosphate into ATP.[135] Several decades of investigation, focusing for a large part on bacterial and bovine systems, have generated profound insights into the molecular mechanisms whereby the mitochondrial ATP synthase operate.[129]

According to current models, the electrochemical gradient built up by the respiratory chain is dissipated as H^+ ions flow between the a subunit and the c-ring, imparting to the latter a relative rotation that is passed to the γ and ε subunits.[136] The rotation of the central stalk (approximate radius = 1 nm) inside a cylindrical lodge formed by the α3β3 hexamer (approximate radius = 5 nm) has been shown to cause conformational changes in F_1 that drive ATP synthesis.[136] Each β subunit contains a nucleotide-binding site (which is localized at the interface with one of the adjacent α subunits) and can assume three discrete conformations: (1) the so-called βDP conformation, which is characterized by an elevated affinity for ADP; (2) the so-called βTP conformation, exhibiting a high affinity for ATP; and (3) the so-called βE conformation, displaying reduced affinity for ATP.[137] Importantly, these three states invariably coexist on an individual F_1 domain, implying that the transition between conformations at distinct α/β interfaces is coordinately regulated.[137]

The central stalk of ATP synthase can rotate up to 700 times/s (depending on temperature, substrate availability and other factors), and each 360° turn results in the synthesis of three ATP molecules.[137] Detailed studies revealed that the γ subunit of the central stalk rotates in discrete 120° steps and that its interaction with a β subunit in the βTP conformation causes the release of ATP from the nucleotide-binding site (that is, the transition to the βE state).[138] Interestingly, it has been suggested that such discrete 120° steps may consist of 30–40° and 80–90° substeps, at least when 'slow' ATPase variants (which release ATP at reduced rates) are concerned.[139] Of note, similar properties could be ascribed neither to hybrid F_1 subunits containing only 1 or 2 slow β subunits,[140] nor to so-called V_1V_O-ATPases,[141,142] variants of F_1F_O-ATPases that generally operate in reverse mode to catalyze the acidification of specific subcellular compartments.[143] Thus, whether the rotation of normal ATPases occurs in discrete substeps $< 120°$ remains to be formally demonstrated.

Irrespective of this unresolved mechanistic issue, ATP synthases appear to catalyze the condensation between ADP and inorganic phosphate by virtue of a functional cooperation between a 'rotor' (formed by the c-ring coupled to the γ, δ and ε subunits) and a 'stator' (consisting of the α3β3 hexamer plus the a, b, d, e, f, g, F6, A6L and O subunits).[128] In this context, special attention should be devoted to the peripheral stalk (composed of the b, d, F6 and O subunits), which links the external surface of F_1 to the a subunit of F_O.[144] This separate substructure appears to have two important roles for ATP synthesis: (1) to counteract the tendency of the α3β3 hexamer to rotate along with the central stalk and the c-ring, and (2) to anchor the a subunit.[128] Interestingly, a and A6L are the only subunits of the F_1F_O-ATPase to be encoded by the mitochondrial genome,[145] and are the last ones to be incorporated into the assembling holoenzyme.[146]

At the 'top' of the F_1 domain, the N-terminal regions of α subunits interact with an OSCP monomer. Electron microscopy-based structural studies of the ATP synthase of *Saccharomyces cerevisiae* demonstrated that the C-terminus of the OSCP is located approximately 90 Å away from the F_1 domain.[147] Of note, the assembly of the latter appears to critically rely on the presence of the ε subunit of the central stalk, which may also be involved in the incorporation of c subunits into the c-ring.[148] These findings indicate that specific subunits of the F_1F_O-ATPase orchestrate the assembly of the catalytically active holoenzyme.

Supramolecular organization of the ATP synthase. Native blue electrophoresis-based experiments coupled to in-gel activity assays have been used to demonstrate that the F_1F_O-ATPase exists not only as a monomer, but also as a dimer and higher-

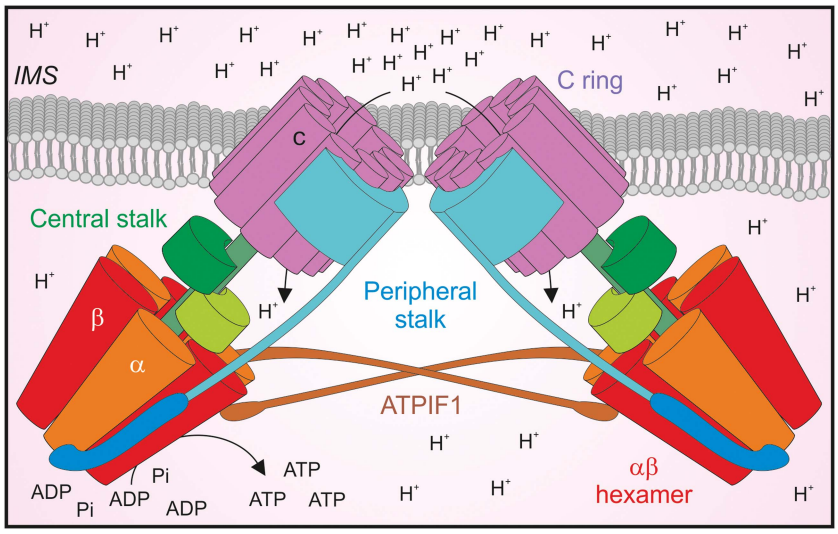

Figure 3. Molecular and supramolecular organization of the mammalian ATP synthase. The mitochondrial ATP synthase consists of a globular domain that protrudes into the mitochondrial matrix (F_1 domain) and an inner mitochondrial membrane-embedded domain (F_O domain), which are interconnected by a central and a peripheral stalk. Mammalian ATP synthases contain 15 different subunits: α, β, γ, δ, ε, a, b, c, d, e, f, g, A6L, F6 and O (also known as OSCP). The F_1 domain consists of three α/β dimers and interacts with both the central stalk (a γ, δ and ε heterotrimer) and the peripheral stalk (which is composed by b, d, F6 and OSCP). The F_O domain involves a ring-shaped oligomer of c subunits stabilized by cardiolipin as well as the a, e, f, g and A6L subunits. Although the a subunit provides a physical dock for the b subunit, A6L appears to bridge F_O to other components of the peripheral stalk. Notably, the ATP synthase form dimers and higher-order oligomer *in cellula*, a process that requires the a, e, g and A6L subunits. The formation of F_1F_O-ATPase dimers is significantly stimulated by ATPIF1, perhaps as this small protein also forms dimers that bridge adjacent F_1 domains. In yeast, ATP synthase monomers engaged in dimeric structures adopt a V-shaped conformation that forms an angle of 86°. IMS, mitochondrial intermembrane space; Pi, inorganic phosphate.

order oligomers (mainly tetramers and hexamers).[149,150] Such oligomers are detectable when mitochondrial proteins are solubilized with mild detergents, such as solutions that contain limited amounts of digitonin.[151] Conversely, when n-dodecyl β-D-maltoside is used for solubilization, most ATP synthase complexes are expected to appear in their monomeric form on native blue gels. Electron cryotomography-based studies demonstrated that the mammalian ATP synthase is arranged in 1 μm-long rows of dimeric supercomplexes that are located at the apex of mitochondrial cristae, a spatial configuration that favors effective ATP synthesis under proton-limited conditions.[152] Electron cryotomography followed by subtomogram averaging also revealed that ATP synthase monomers from *S. cerevisiae* form symmetrical V-shaped dimers with an angle of 86°.[153] Specific components of the yeast F_1F_O-ATPase (that is, the e and g subunits as well as the first transmembrane helix of subunit 4) appears to be required for the formation of ATP synthase dimers.[153–155] The critical involvement of the e and g subunits in the dimerization of the F_1F_O-ATPase has also been documented in the mammalian system.[156,157] Moreover, the dimerization of the mammalian F_1F_O-ATPase reportedly requires the a and A6L subunits.[146]

Of note, it appears that ATP synthase dimers contribute to the maintenance of the mitochondrial morphology as they promote the formation of highly curved cristae ridges.[153] In line with this notion, as *Podospora anserina* (a filamentous fungus) ages, ATP synthase dimers dissociate into monomers, a degenerative process that is associated with the loss of mitochondrial cristae.[158] The ATPase inhibitory factor 1 (ATPIF1), a heat-stable protein that inhibits ATP synthesis as it stimulates F_1F_O-ATPase to operate in reverse mode,[159,160] has also been implicated in the dimerization of the ATP synthase.[161] Crystallographic and electron microscopy-based studies suggest indeed that dimeric ATPIF1 may stabilize ATPase dimers at the level of F_1 domains.[157,161]

Importantly, the F_1F_O-ATPase synthesizes ATP from ADP and inorganic phosphate only in the presence of an adequate proton-motive force (*pmf*). In mitochondria, such a *pmf* is generated by respiratory chain complexes, establishing across the inner mitochondrial membrane the proton concentration gradient (ΔpH) that underlies the $\Delta\psi_m$.[162] Conversely, in the absence of an adequate *pmf*, F_1 avidly hydrolyzes ATP.[162] However, this mechanism accounts for the lethal effects of MOMP and MPT to a very limited extent.[1,11] Indeed, in response to declines in the mitochondrial *pmf* (such as those induced by hypoxia), ATPIF1 inhibits the hydrolytic activity of F_1, hence avoiding a potentially lethal drop in intracellular ATP levels.[163,164] In this context, it should be emphasized that the F_1F_O-ATPase would consume ATP of cytosolic origin only (1) if the $\Delta\psi_m$ exceeded the so-called 'reversal potential' of ANT, that is, the value of $\Delta\psi_m$ at which there is no net exchange of ADP and ATP across the inner mitochondrial membrane; and (2) ATP in the mitochondrial matrix could not be provided by substrate-level phosphorylation.[165–170] ATPIF1 has recently been shown to limit the translocation of BAX to the outer mitochondrial membrane under pro-apoptotic conditions, presumably as it prevents mitochondrial remodeling.[171] These findings lend further support to the notion that the molecular machineries that regulate mitochondrial dynamics, MOMP and MPT, engage in an intimate, mutually regulatory crosstalk.[172–174]

The mitochondrial ATP synthase gives the 'wedding ring' to the PTPC. Several parameters that alter the threshold for the induction of MPT have also been shown to regulate the catalytic activity of the ATP synthase.[175] First, the hydrolytic activity of the F_1F_O-ATPase is strongly inhibited by the concurrent binding of ADP and Mg^{2+}, two potent MPT inhibitors, to its catalytic site, a situation known as Mg-ADP block.[162] ADP and Mg^{2+} ions are required for ATP synthesis and limit the catabolic activity of the ATP synthase in a non-competitive manner that differs from simple product inhibition.[176–180] Of note, the Mg-ADP block can be resolved by an increase in *pmf*, expelling Mg^{2+} ions and ADP from the inhibitory site.[162,181] Inorganic phosphate, a prominent inducer of MPT, has also been proposed to relieve the Mg-ADP block.[162,182,183] Thus, inorganic phosphate concentrations >5 mM

robustly activate the hydrolytic activity of the F_1F_O-ATPase.[179,184,185] Second, similar to ANT,[186] the ATP synthase is sensitive to the oxidation of specific cysteine residues (that is, Cys294 and Cys103 in the α and γ subunit, respectively), resulting in the formation of an inter-subunit, inhibitory disulfide bridge.[187] Moreover, the catalytic activity of the F_1F_O-ATPase is influenced by $\Delta\psi_m$ and pH,[162] which also affect the sensitivity of the PTPC to MPT inducers.[188–190]

Similar to the PTPC, the ATP synthase engages in physical and functional interactions with a large panel of mitochondrial proteins.[191] In particular, the F_1F_O-ATPase has been shown to form supercomplexes with ANT family members and PiC (both of which have been involved in MPT and both of which contain oxidative stress-sensitive thiol residues),[192,193] the so-called ATP synthasomes.[191,194–196] According to current models, the topological arrangement of ATP synthasomes would maximize the efficiency of ATP production and export.[191,194–196] Moreover, the F_1F_O-ATPase reportedly binds CYPD via the peripheral stalk, in particular, OSCP and subunit d.[197] This CsA-sensitive interaction reduces both the synthetic and hydrolytic activity of the ATP synthase.[197] However, the F_1F_O-ATPase-modulatory functions of CYPD only influence the intramitochondrial pool of adenine nucleotides, leaving its cytoplasmic counterpart unaffected.[198] Finally, several members of the Bcl-2 protein family appear to interact, physically or functionally, with the ATP synthase.[199–201] In particular, BCL-X_L, which is known to inhibit MPT upon binding to VDAC1,[111,112] reportedly binds the F_1F_O-ATPase, hence enhancing its synthetic activity.[199,200] Along similar lines, an amino-terminally truncated version of MCL-1 that localizes to the mitochondrial matrix (as opposed to the full-length MCL-1, which inserts into the outer mitochondrial membrane) not only promotes the activity of the mitochondrial respiratory chain, hence increasing the $\Delta\psi_m$ and stimulating ATP production, but also favors the oligomeric state of ATP synthase and thus preserves mitochondrial ultrastructure.[201] This said, whether MCL-1 physically interacts with one or more F_1F_O-ATPase subunits or whether its effects on the oligomerization of ATP synthase are indirect, has not yet been clarified.

Pharmacological data also suggest a link between the F_1F_O-ATPase and MPT. For instance, oligomycin, which inhibits the catalytic activity of the ATP synthase upon binding to the F_O subunit,[202] has been shown to block MPT as induced by erucylphosphohomocholine (an antineoplastic agent also known as erufosine), as well as by BAX- and tumor necrosis factor receptor 1-activating conditions.[119,203–205] Of note, similar MPT-inhibitory effects could not be ascribed to piceatannol, which inhibits the F_1 domain of ATP synthase.[205] Taken together, these findings suggest that the ATP synthase (in particular, the F_O domain) may have a central role in MPT.

In 2013, the suspicion about the central implication of the F_1F_O-ATPase in MPT crystallized as Paolo Bernardi's group proposed that the pore-forming unit of the PTPC would consist of ATP synthase dimers.[206,207] However, the demonstration that ρ° cells, which lack mitochondrial DNA, retain a functional PTPC argues against this model.[208] Indeed, in line with the fact that the dimerization of the F_1F_O-ATPase requires the a and A6L subunits (which are encoded by the mitochondrial genome), ρ° cells contains (highly unstable) ATP synthase dimers at extremely low levels.[146] Moreover, the dimerization of ATP synthase, which is promoted by ATPIF1,[161] has been associated with MPT-inhibitory and cytoprotective effects in several experimental paradigms.[159] Conversely, the relative proportion of F_1F_O-ATPase dimers over monomers decreases in aged cells, correlating with increasing rates of cell death.[158] Of note, such a transition between the dimeric and monomeric form of the ATP synthase appears to be stimulated by CYPD,[158] reinforcing the notion that F_1F_O-ATPase oligomers mediate cytoprotective, rather than cytotoxic, effects.

Among the components of the F_O domain, the highly conserved a, b and c subunits are sufficient to allow for the translocation of protons across lipid bilayers.[209] The c subunit binds Ca^{2+} and has actually been ascribed with pore-forming properties.[210,211] Moreover, a peptide displaying a high degree of similarity to the c subunit has been proposed to operate as a PTPC regulator.[212,213] Driven by these observations and by the fact that the a subunit appears to be dispensable for MPT,[208] we recently set out to determine the contribution of the c subunit to the PTPC.[214] We found that the transient depletion of the c subunit (by means of ATP5G-targeting small-interfering RNAs) prevents the induction of MPT by Ca^{2+} and oxidants, while its overexpression markedly promotes MPT (and hence results in some extent of cell death *per se*).[214] Of note, the MPT-regulatory effects of depleting the c subunit were not influenced by the metabolic profile (glycolytic or respiratory) of the cells, nor were they mimicked by the transient depletion of the α subunit (ATP5A1). Moreover, the temporary depletion of the c subunit did not affect mitochondrial ATP levels,[214] indicating that the effects on MPT that we observed did not reflect changes in the availability of adenine nucleotides. Subsequent work by another group demonstrated that the addition of purified c subunits to isolated mitochondria provokes MPT depending on its own phosphorylation state.[215] However, the possibility that c-rings may exist in physiological conditions independently of other components of the ATP synthase has not yet been addressed.

CONCLUSIONS AND PERSPECTIVES

In spite of an intense wave of investigation, the precise molecular composition of the PTPC remains to be unveiled. As MPT is triggered by conditions that promote protein unfolding, it has also been proposed that the PTPC would just assemble by the unspecific interaction of denatured proteins, (virtually) irrespective of their identity.[1,3,216] The evidence in support of this theory, however, is rather circumstantial. The study of the PTPC is actually problematic, for at least two reasons. First, several (presumed) core PTPC components exist in multiple isoforms, which significantly complicates the generation of adequate knockout models.[53,54] Second, many proteins that have been involved in MPT exert key vital functions, a situation that is incompatible not only with the generation of murine knockout models, but also with strategies of stable cellular depletion.[217,218] This latter issue could be circumvented by knock-in strategies aimed at replacing the wild-type protein with a mutant that is selectively impaired in its capacity to modulate cell death, an approach that was successful for the central MOMP regulator cytochrome *c*.[219]

Here, we propose that the ATP synthase has a central role in MPT, based on the following observations: (1) the F_1F_O-ATPase and the PTPC share several pharmacological and endogenous modulators; (2) the F_1F_O-ATPase interacts with several MPT regulators, including ANT, PiC and CYPD; (3) the genetic modulation of the levels of the c subunit (the sole ATP synthase component with confirmed conductive capacity) influences the propensity of mitochondria to undergo MPT, *in vitro* and *in cellula*. As it stands, it seems premature to identify the c subunit of the F_1F_O-ATPase as the mysterious pore-forming component of PTPC. Perhaps, the ATP synthasome simply operates as a regulatory dock for another, hitherto uncharacterized protein that disrupts the physical integrity of the inner mitochondrial membrane. Further studies based on robust genetic models will have to formally address these possibilities.

ABBREVIATIONS

ANT, adenine nucleotide translocase; ATPIF1, ATPase inhibitory factor 1; CKMT1, creatine kinase, mitochondrial 1; CsA, cyclosporine A; CYPD, cyclophilin D; $\Delta\psi_m$, mitochondrial transmembrane

potential; GSK3β, glycogen synthase kinase 3β; HK, hexokinase; MOMP, mitochondrial outer membrane permeabilization; MPT, mitochondrial permeability transition; OSCP, oligomycin sensitivity-conferring protein; PKCε, protein kinase Cε; *pmf*, proton-motive force; PTPC, permeability transition pore complex; TSPO, translocator protein (18 kDa); VDAC, voltage-dependent anion channel

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007; **87**: 99–163.
2 Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV *et al.* Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ 2012*, **19**: 107–120.
3 Brenner C, Grimm S. The permeability transition pore complex in cancer cell death. *Oncogene* 2006; **25**: 4744–4756.
4 Hunter DR, Haworth RA. The Ca2+-induced membrane transition in mitochondria. I. The protective mechanisms. *Arch Biochem Biophys* 1979; **195**: 453–459.
5 Zamzami N, Marchetti P, Castedo M, Decaudin D, Macho A, Hirsch T *et al.* Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. *J Exp Med* 1995; **182**: 367–377.
6 Zamzami N, Marchetti P, Castedo M, Zanin C, Vayssiere JL, Petit PX *et al.* Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death in vivo. *J Exp Med* 1995; **181**: 1661–1672.
7 Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 2011; **333**: 1109–1112.
8 Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat Rev Mol Cell Biol* 2010; **11**: 700–714.
9 Bonora M, Patergnani S, Rimessi A, De Marchi E, Suski JM, Bononi A *et al.* ATP synthesis and storage. *Purinergic Signal* 2012; **8**: 343–357.
10 Galluzzi L, Kepp O, Kroemer G. Mitochondria: master regulators of danger signalling. *Nat Rev Mol Cell Biol* 2012; **13**: 780–788.
11 Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat Rev Mol Cell Biol* 2010; **11**: 621–632.
12 Munoz-Pinedo C, Guio-Carrion A, Goldstein JC, Fitzgerald P, Newmeyer DD, Green DR. Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. *Proc Natl Acad Sci USA* 2006; **103**: 11573–11578.
13 Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ 2009*, **16**: 3–11.
14 Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008; **9**: 231–241.
15 Leist M, Single B, Castoldi AF, Kuhnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* 1997; **185**: 1481–1486.
16 Mannick JB, Schonhoff C, Papeta N, Ghafourifar P, Szibor M, Fang K *et al.* S-nitrosylation of mitochondrial caspases. *J Cell Biol* 2001; **154**: 1111–1116.
17 Jost PJ, Grabow S, Gray D, McKenzie MD, Nachbur U, Huang DC *et al.* XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 2009; **460**: 1035–1039.
18 Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C *et al.* Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* 2011; **471**: 363–367.
19 Hirsch T, Marchetti P, Susin SA, Dallaporta B, Zamzami N, Marzo I *et al.* The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. *Oncogene* 1997; **15**: 1573–1581.
20 Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J *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* 2005; **102**: 12005–12010.
21 Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA *et al.* Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 2005; **434**: 658–662.
22 Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H *et al.* Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005; **434**: 652–658.
23 Whelan RS, Konstantinidis K, Wei AC, Chen Y, Reyna DE, Jha S *et al.* Bax regulates primary necrosis through mitochondrial dynamics. *Proc Natl Acad Sci USA* 2012; **109**: 6566–6571.
24 Linkermann A, Brasen JH, Darding M, Jin MK, Sanz AB, Heller JO *et al.* Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 2013; **110**: 12024–12029.
25 Thomas B, Banerjee R, Starkova NN, Zhang SF, Calingasan NY, Yang L *et al.* Mitochondrial permeability transition pore component cyclophilin D distinguishes nigrostriatal dopaminergic death paradigms in the MPTP mouse model of Parkinson's disease. *Antioxid Redox Signal* 2012; **16**: 855–868.
26 Ramachandran A, Lebofsky M, Baines CP, Lemasters JJ, Jaeschke H. Cyclophilin D deficiency protects against acetaminophen-induced oxidant stress and liver injury. *Free Radic Res* 2011; **45**: 156–164.
27 LoGuidice A, Ramirez-Alcantara V, Proli A, Gavillet B, Boelsterli UA. Pharmacologic targeting or genetic deletion of mitochondrial cyclophilin D protects from NSAID-induced small intestinal ulceration in mice. *Toxicol Sci* 2010; **118**: 276–285.
28 Haouzi D, Cohen I, Vieira HL, Poncet D, Boya P, Castedo M *et al.* Mitochondrial permeability transition as a novel principle of hepatorenal toxicity in vivo. *Apoptosis* 2002; **7**: 395–405.
29 Fujimoto K, Chen Y, Polonsky KS, Dorn GW2nd. Targeting cyclophilin D and the mitochondrial permeability transition enhances beta-cell survival and prevents diabetes in Pdx1 deficiency. *Proc Natl Acad Sci USA* 2010; **107**: 10214–10219.
30 Palma E, Tiepolo T, Angelin A, Sabatelli P, Maraldi NM, Basso E *et al.* Genetic ablation of cyclophilin D rescues mitochondrial defects and prevents muscle apoptosis in collagen VI myopathic mice. *Hum Mol Genet* 2009; **18**: 2024–2031.
31 Millay DP, Sargent MA, Osinska H, Baines CP, Barton ER, Vuagniaux G *et al.* Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis attenuates muscular dystrophy. *Nat Med* 2008; **14**: 442–447.
32 Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O *et al.* Molecular mechanisms of cisplatin resistance. *Oncogene* 2012; **31**: 1869–1883.
33 Chipuk JE, Fisher JC, Dillon CP, Kriwacki RW, Kuwana T, Green DR. Mechanism of apoptosis induction by inhibition of the anti-apoptotic BCL-2 proteins. *Proc Natl Acad Sci USA* 2008; **105**: 20327–20332.
34 Peng R, Tong JS, Li H, Yue B, Zou F, Yu J *et al.* Targeting Bax interaction sites reveals that only homo-oligomerization sites are essential for its activation. *Cell Death Differ* 2013; **20**: 744–754.
35 Galluzzi L, Blomgren K, Kroemer G. Mitochondrial membrane permeabilization in neuronal injury. *Nat Rev Neurosci* 2009; **10**: 481–494.
36 Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov* 2010; **9**: 447–464.
37 Galluzzi L, Larochette N, Zamzami N, Kroemer G. Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene* 2006; **25**: 4812–4830.
38 Martel C, Huynh le H, Garnier A, Ventura-Clapier R, Brenner C. Inhibition of the mitochondrial permeability transition for cytoprotection: direct versus indirect Mechanisms. *Biochem Res Int* 2012; **2012**: 213403.
39 Kepp O, Galluzzi L, Lipinski M, Yuan J, Kroemer G. Cell death assays for drug discovery. *Nat Rev Drug Discov* 2011; **10**: 221–237.
40 Kahan BD. Individuality: the barrier to optimal immunosuppression. *Nat Rev Immunol* 2003; **3**: 831–838.
41 Borner C, Andrews DW. The apoptotic pore on mitochondria: are we breaking through or still stuck? *Cell Death Differ* 2014; **21**: 187–191.

42 Szabo I, Zoratti M. The mitochondrial megachannel is the permeability transition pore. *J Bioenerg Biomembr* 1992; **24**: 111–117.
43 Szabo I, Bernardi P, Zoratti M. Modulation of the mitochondrial megachannel by divalent cations and protons. *J Biol Chem* 1992; **267**: 2940–2946.
44 Szabo I, Zoratti M. The mitochondrial permeability transition pore may comprise VDAC molecules. I. Binary structure and voltage dependence of the pore. *FEBS Lett* 1993; **330**: 201–205.
45 Szabo I, De Pinto V, Zoratti M. The mitochondrial permeability transition pore may comprise VDAC molecules. II. The electrophysiological properties of VDAC are compatible with those of the mitochondrial megachannel. *FEBS Lett* 1993; **330**: 206–210.
46 McEnery MW, Snowman AM, Trifiletti RR, Snyder SH. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proc Natl Acad Sci USA* 1992; **89**: 3170–3174.
47 Beutner G, Ruck A, Riede B, Welte W, Brdiczka D. Complexes between kinases, mitochondrial porin and adenylate translocator in rat brain resemble the permeability transition pore. *FEBS Lett* 1996; **396**: 189–195.
48 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* 1998; **1368**: 7–18.
49 Crompton M, Virji S, Ward JM. Cyclophilin-D binds strongly to complexes of the voltage-dependent anion channel and the adenine nucleotide translocase to form the permeability transition pore. *Eur J Biochem* 1998; **258**: 729–735.
50 Tanveer A, Virji S, Andreeva L, Totty NF, Hsuan JJ, Ward JM *et al.* Involvement of cyclophilin D in the activation of a mitochondrial pore by Ca2+ and oxidant stress. *Eur J Biochem* 1996; **238**: 166–172.
51 Halestrap AP, Davidson AM. Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J* 1990; **268**: 153–160.
52 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* 1998; **426**: 97–101.
53 Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP *et al.* The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 2004; **427**: 461–465.
54 Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* 2007; **9**: 550–555.
55 Galluzzi L, Kroemer G. Mitochondrial apoptosis without VDAC. *Nat Cell Biol* 2007; **9**: 487–489.
56 Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J Biol Chem* 2005; **280**: 18558–18561.
57 Dolce V, Scarcia P, Iacopetta D, Palmieri F. A fourth ADP/ATP carrier isoform in man: identification, bacterial expression, functional characterization and tissue distribution. *FEBS Lett* 2005; **579**: 633–637.
58 Zamora M, Granell M, Mampel T, Vinas O. Adenine nucleotide translocase 3 (ANT3) overexpression induces apoptosis in cultured cells. *FEBS Lett* 2004; **563**: 155–160.
59 Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science* 2003; **301**: 513–517.
60 Desagher S, Osen-Sand A, Nichols A, Eskes R, Montessuit S, Lauper S *et al.* Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. *J Cell Biol* 1999; **144**: 891–901.
61 Tedeschi H, Hegarty HJ, James JM. Osmotic reversal of phosphate-induced mitochondrial swelling. *Biochim Biophys Acta* 1965; **104**: 612–615.
62 Palmieri F. The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflugers Arch* 2004; **447**: 689–709.
63 Hagen T, Lagace CJ, Modica-Napolitano JS, Aprille JR. Permeability transition in rat liver mitochondria is modulated by the ATP-Mg/Pi carrier. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G274–G281.
64 Traba J, Del Arco A, Duchen MR, Szabadkai G, Satrustegui J. SCaMC-1 promotes cancer cell survival by desensitizing mitochondrial permeability transition via ATP/ADP-mediated matrix Ca(2+) buffering. *Cell Death Differ* 2012; **19**: 650–660.
65 Poncet D, Pauleau AL, Szabadkai G, Vozza A, Scholz SR, Le Bras M *et al.* Cytopathic effects of the cytomegalovirus-encoded apoptosis inhibitory protein vMIA. *J Cell Biol* 2006; **174**: 985–996.
66 Galluzzi L, Brenner C, Morselli E, Touat Z, Kroemer G. Viral control of mitochondrial apoptosis. *PLoS Pathog* 2008; **4**: e1000018.
67 Leung AW, Varanyuwatana P, Halestrap AP. The mitochondrial phosphate carrier interacts with cyclophilin D and may play a key role in the permeability transition. *J Biol Chem* 2008; **283**: 26312–26323.
68 Alcala S, Klee M, Fernandez J, Fleischer A, Pimentel-Muinos FX. A high-throughput screening for mammalian cell death effectors identifies the mitochondrial phosphate carrier as a regulator of cytochrome c release. *Oncogene* 2008; **27**: 44–54.
69 Schroers A, Kramer R, Wohlrab H. The reversible antiport-uniport conversion of the phosphate carrier from yeast mitochondria depends on the presence of a single cysteine. *J Biol Chem* 1997; **272**: 10558–10564.
70 Varanyuwatana P, Halestrap AP. The roles of phosphate and the phosphate carrier in the mitochondrial permeability transition pore. *Mitochondrion* 2012; **12**: 120–125.
71 Pauleau AL, Galluzzi L, Scholz SR, Larochette N, Kepp O, Kroemer G. Unexpected role of the phosphate carrier in mitochondrial fragmentation. *Cell Death Differ* 2008; **15**: 616–618.
72 Verrier F, Deniaud A, Lebras M, Metivier D, Kroemer G, Mignotte B *et al.* Dynamic evolution of the adenine nucleotide translocase interactome during chemotherapy-induced apoptosis. *Oncogene* 2004; **23**: 8049–8064.
73 Mukhin AG, Papadopoulos V, Costa E, Krueger KE. Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proc Natl Acad Sci USA* 1989; **86**: 9813–9816.
74 Pastorino JG, Simbula G, Gilfor E, Hoek JB, Farber JL. Protoporphyrin IX, an endogenous ligand of the peripheral benzodiazepine receptor, potentiates induction of the mitochondrial permeability transition and the killing of cultured hepatocytes by rotenone. *J Biol Chem* 1994; **269**: 31041–31046.
75 Hirsch T, Decaudin D, Susin SA, Marchetti P, Larochette N, Resche-Rigon M *et al.* PK11195, a ligand of the mitochondrial benzodiazepine receptor, facilitates the induction of apoptosis and reverses Bcl-2-mediated cytoprotection. *Exp Cell Res* 1998; **241**: 426–434.
76 Chelli B, Falleni A, Salvetti F, Gremigni V, Lucacchini A, Martini C. Peripheral-type benzodiazepine receptor ligands: mitochondrial permeability transition induction in rat cardiac tissue. *Biochem Pharmacol* 2001; **61**: 695–705.
77 Azarashvili T, Grachev D, Krestinina O, Evtodienko Y, Yurkov I, Papadopoulos V *et al.* The peripheral-type benzodiazepine receptor is involved in control of Ca2+-induced permeability transition pore opening in rat brain mitochondria. *Cell Calcium* 2007; **42**: 27–39.
78 Klaffschenkel RA, Waidmann M, Northoff H, Mahmoud AA, Lembert N. PK11195, a specific ligand of the peripheral benzodiazepine receptor, may protect pancreatic beta-cells from cytokine-induced cell death. *Artif Cells Blood Substit Immobil Biotechnol* 2012; **40**: 56–61.
79 Kugler W, Veenman L, Shandalov Y, Leschiner S, Spanier I, Lakomek M *et al.* Ligands of the mitochondrial 18 kDa translocator protein attenuate apoptosis of human glioblastoma cells exposed to erucylphosphohomocholine. *Cell Oncol* 2008; **30**: 435–450.
80 Shargorodsky L, Veenman L, Caballero B, Pe'er Y, Leschiner S, Bode J *et al.* The nitric oxide donor sodium nitroprusside requires the 18 kDa Translocator Protein to induce cell death. *Apoptosis* 2012; **17**: 647–665.
81 Campanella M, Szabadkai G, Rizzuto R. Modulation of intracellular Ca2+ signalling in HeLa cells by the apoptotic cell death enhancer PK11195. *Biochem Pharmacol* 2008; **76**: 1628–1636.
82 Decaudin D, Castedo M, Nemati F, Beurdeley-Thomas A, De Pinieux G, Caron A *et al.* Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells in vitro and in vivo. *Cancer Res* 2002; **62**: 1388–1393.
83 Gonzalez-Polo RA, Carvalho G, Braun T, Decaudin D, Fabre C, Larochette N *et al.* PK11195 potently sensitizes to apoptosis induction independently from the peripheral benzodiazepin receptor. *Oncogene* 2005; **24**: 7503–7513.
84 Wallimann T, Dolder M, Schlattner U, Eder M, Hornemann T, O'Gorman E *et al.* Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. *Biofactors* 1998; **8**: 229–234.
85 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* 2003; **278**: 17760–17766.
86 Wilson JE. Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. *J Exp Biol* 2003; **206**: 2049–2057.
87 Pastorino JG, Hoek JB. Regulation of hexokinase binding to VDAC. *J Bioenerg Biomembr* 2008; **40**: 171–182.
88 Pastorino JG, Hoek JB. Hexokinase II: the integration of energy metabolism and control of apoptosis. *Curr Med Chem* 2003; **10**: 1535–1551.
89 Galluzzi L, Kepp O, Tajeddine N, Kroemer G. Disruption of the hexokinase-VDAC complex for tumor therapy. *Oncogene* 2008; **27**: 4633–4635.
90 Goldin N, Arzoine L, Heyfets A, Israelson A, Zaslavsky Z, Bravman T *et al.* Methyl jasmonate binds to and detaches mitochondria-bound hexokinase. *Oncogene* 2008; **27**: 4636–4643.

91 Arzoine L, Zilberberg N, Ben-Romano R, Shoshan-Barmatz V. Voltage-dependent anion channel 1-based peptides interact with hexokinase to prevent its anti-apoptotic activity. *J Biol Chem* 2009; **284**: 3946–3955.
92 Smeele KM, Southworth R, Wu R, Xie C, Nederlof R, Warley A *et al.* Disruption of hexokinase II-mitochondrial binding blocks ischemic preconditioning and causes rapid cardiac necrosis. *Circ Res* 2011; **108**: 1165–1169.
93 Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G. Metabolic targets for cancer therapy. *Nat Rev Drug Discov* 2013; **12**: 829–846.
94 Sun L, Shukair S, Naik TJ, Moazed F, Ardehali H. Glucose phosphorylation and mitochondrial binding are required for the protective effects of hexokinases I and II. *Mol Cell Biol* 2008; **28**: 1007–1017.
95 Chiara F, Castellaro D, Marin O, Petronilli V, Brusilow WS, Juhaszova M *et al.* Hexokinase II detachment from mitochondria triggers apoptosis through the permeability transition pore independent of voltage-dependent anion channels. *PLoS ONE* 2008; **3**: e1852.
96 Schindler A, Foley E. Hexokinase 1 blocks apoptotic signals at the mitochondria. *Cell Signal* 2013; **25**: 2685–2692.
97 Pastorino JG, Hoek JB, Shulga N. Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity. *Cancer Res* 2005; **65**: 10545–10554.
98 Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL *et al.* Protein kinase Cepsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. *Circ Res* 2003; **92**: 873–880.
99 Takuma K, Phuagphong P, Lee E, Mori K, Baba A, Matsuda T. Anti-apoptotic effect of cGMP in cultured astrocytes: inhibition by cGMP-dependent protein kinase of mitochondrial permeable transition pore. *J Biol Chem* 2001; **276**: 48093–48099.
100 Das S, Wong R, Rajapakse N, Murphy E, Steenbergen C. Glycogen synthase kinase 3 inhibition slows mitochondrial adenine nucleotide transport and regulates voltage-dependent anion channel phosphorylation. *Circ Res* 2008; **103**: 983–991.
101 Nishihara M, Miura T, Miki T, Tanno M, Yano T, Naitoh K *et al.* Modulation of the mitochondrial permeability transition pore complex in GSK-3beta-mediated myocardial protection. *J Mol Cell Cardiol* 2007; **43**: 564–570.
102 Chiara F, Gambalunga A, Sciacovelli M, Nicolli A, Ronconi L, Fregona D *et al.* Chemotherapeutic induction of mitochondrial oxidative stress activates GSK--3alpha/beta and Bax, leading to permeability transition pore opening and tumor cell death. *Cell Death Dis* 2012; **3**: e444.
103 Rasola A, Sciacovelli M, Chiara F, Pantic B, Brusilow WS, Bernardi P. Activation of mitochondrial ERK protects cancer cells from death through inhibition of the permeability transition. *Proc Natl Acad Sci USA* 2010; **107**: 726–731.
104 Korzick DH, Kostyak JC, Hunter JC, Saupe KW. Local delivery of PKCepsilon-activating peptide mimics ischemic preconditioning in aged hearts through GSK-3beta but not F1-ATPase inactivation. *Am J Physiol Heart Circ Physiol* 2007; **293**: H2056–H2063.
105 Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW *et al.* Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest* 2004; **113**: 1535–1549.
106 Pediaditakis P, Kim JS, He L, Zhang X, Graves LM, Lemasters JJ. Inhibition of the mitochondrial permeability transition by protein kinase A in rat liver mitochondria and hepatocytes. *Biochem J* 2010; **431**: 411–421.
107 Juhaszova M, Zorov DB, Yaniv Y, Nuss HB, Wang S, Sollott SJ. Role of glycogen synthase kinase-3beta in cardioprotection. *Circ Res* 2009; **104**: 1240–1252.
108 Marzo I, Brenner C, Zamzami N, Jurgensmeier JM, Susin SA, Vieira HL *et al.* Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. *Science* 1998; **281**: 2027–2031.
109 Brenner C, Cadiou H, Vieira HL, Zamzami N, Marzo I, Xie Z *et al.* Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator. *Oncogene* 2000; **19**: 329–336.
110 Zamzami N, El Hamel C, Maisse C, Brenner C, Munoz-Pinedo C, Belzacq AS *et al.* Bid acts on the permeability transition pore complex to induce apoptosis. *Oncogene* 2000; **19**: 6342–6350.
111 Shimizu S, Narita M, Tsujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature* 1999; **399**: 483–487.
112 Vander Heiden MG, Chandel NS, Schumacker PT, Thompson CB. Bcl-xL prevents cell death following growth factor withdrawal by facilitating mitochondrial ATP/ADP exchange. *Mol Cell* 1999; **3**: 159–167.
113 Roy SS, Madesh M, Davies E, Antonsson B, Danial N, Hajnoczky G. Bad targets the permeability transition pore independent of Bax or Bak to switch between Ca2+-dependent cell survival and death. *Mol Cell* 2009; **33**: 377–388.
114 Arbel N, Ben-Hail D, Shoshan-Barmatz V. Mediation of the antiapoptotic activity of Bcl-xL protein upon interaction with VDAC1 protein. *J Biol Chem* 2012; **287**: 23152–23161.
115 Malia TJ, Wagner G. NMR structural investigation of the mitochondrial outer membrane protein VDAC and its interaction with antiapoptotic Bcl-xL. *Biochemistry* 2007; **46**: 514–525.
116 Todt F, Cakir Z, Reichenbach F, Youle RJ, Edlich F. The C-terminal helix of Bcl-x(L) mediates Bax retrotranslocation from the mitochondria. *Cell Death Differ* 2013; **20**: 333–342.
117 Vaseva AV, Marchenko ND, Ji K, Tsirka SE, Holzmann S, Moll UM. p53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell* 2012; **149**: 1536–1548.
118 Rufini A, Tucci P, Celardo I, Melino G. Senescence and aging: the critical roles of p53. *Oncogene* 2013; **32**: 5129–5143.
119 Narita M, Shimizu S, Ito T, Chittenden T, Lutz RJ, Matsuda H *et al.* Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. *Proc Natl Acad Sci USA* 1998; **95**: 14681–14686.
120 Karch J, Kwong JQ, Burr AR, Sargent MA, Elrod JW, Peixoto PM *et al.* Bax and Bak function as the outer membrane component of the mitochondrial permeability pore in regulating necrotic cell death in mice. *Elife* 2013; **2**: e00772.
121 Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T *et al.* BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science* 2003; **300**: 135–139.
122 Marchi S, Patergnani S, Pinton P. The endoplasmic reticulum-mitochondria connection: one touch, multiple functions. *Biochim Biophys Acta* 2014; **1837**: 461–469.
123 Marchi S, Pinton P. The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. *J Physiol* 2014; **592**: 829–839 in press.
124 Patergnani S, Suski JM, Agnoletto C, Bononi A, Bonora M, De Marchi E *et al.* Calcium signaling around mitochondria associated membranes (MAMs). *Cell Commun Signal* 2011; **9**: 19.
125 Giorgi C, Baldassari F, Bononi A, Bonora M, De Marchi E, Marchi S *et al.* Mitochondrial Ca(2+) and apoptosis. *Cell Calcium* 2012; **52**: 36–43.
126 Giorgi C, Ito K, Lin HK, Santangelo C, Wieckowski MR, Lebiedzinska M *et al.* PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science* 2010; **330**: 1247–1251.
127 Yoshida M, Muneyuki E, Hisabori T. ATP synthase--a marvellous rotary engine of the cell. *Nat Rev Mol Cell Biol* 2001; **2**: 669–677.
128 Devenish RJ, Prescott M, Rodgers AJ. The structure and function of mitochondrial F1F0-ATP synthases. *Int Rev Cell Mol Biol* 2008; **267**: 1–58.
129 Okuno D, Iino R, Noji H. Rotation and structure of FoF1-ATP synthase. *J Biochem* 2011; **149**: 655–664.
130 Rubinstein JL, Walker JE, Henderson R. Structure of the mitochondrial ATP synthase by electron cryomicroscopy. *EMBO J* 2003; **22**: 6182–6192.
131 Jonckheere AI, Smeitink JA, Rodenburg RJ. Mitochondrial ATP synthase: architecture, function and pathology. *J Inherit Metab Dis* 2012; **35**: 211–225.
132 Swanljung P, Frigeri L, Ohlson K, Ernster L. Studies on the activation of purified mitochondrial ATPase by phospholipids. *Biochim Biophys Acta* 1973; **305**: 519–533.
133 Lightowlers RN, Howitt SM, Hatch L, Gibson F, Cox G. The proton pore in the *Escherichia coli* F0F1-ATPase: substitution of glutamate by glutamine at position 219 of the alpha-subunit prevents F0-mediated proton permeability. *Biochim Biophys Acta* 1988; **933**: 241–248.
134 Stephens AN, Nagley P, Devenish RJ. Each yeast mitochondrial F1F0-ATP synthase complex contains a single copy of subunit 8. *Biochim Biophys Acta* 2003; **1607**: 181–189.
135 Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* 1961; **191**: 144–148.
136 Noji H, Yasuda R, Yoshida M, Kinosita K Jr. Direct observation of the rotation of F1-ATPase. *Nature* 1997; **386**: 299–302.
137 von Ballmoos C, Wiedenmann A, Dimroth P. Essentials for ATP synthesis by F1F0 ATP synthases. *Annu Rev Biochem* 2009; **78**: 649–672.
138 Yasuda R, Noji H, Kinosita KJr., Yoshida M. F1-ATPase is a highly efficient molecular motor that rotates with discrete 120 degree steps. *Cell* 1998; **93**: 1117–1124.
139 Shimabukuro K, Muneyuki E, Yoshida M. An alternative reaction pathway of F1-ATPase suggested by rotation without 80 degrees/40 degrees substeps of a sluggish mutant at low ATP. *Biophys J* 2006; **90**: 1028–1032.
140 Ariga T, Masaike T, Noji H, Yoshida M. Stepping rotation of F(1)-ATPase with one, two, or three altered catalytic sites that bind ATP only slowly. *J Biol Chem* 2002; **277**: 24870–24874.
141 Minagawa Y, Ueno H, Hara M, Ishizuka-Katsura Y, Ohsawa N, Terada T *et al.* Basic properties of rotary dynamics of the molecular motor Enterococcus hirae V1-ATPase. *J Biol Chem* 2013; **288**: 32700–32707.

142 Arai S, Saijo S, Suzuki K, Mizutani K, Kakinuma Y, Ishizuka-Katsura Y *et al.* Rotation mechanism of Enterococcus hirae V1-ATPase based on asymmetric crystal structures. *Nature* 2013; **493**: 703–707.
143 Forgac M. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol* 2007; **8**: 917–929.
144 Walker JE, Dickson VK. The peripheral stalk of the mitochondrial ATP synthase. *Biochim Biophys Acta* 2006; **1757**: 286–296.
145 Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J *et al.* Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–465.
146 Wittig I, Meyer B, Heide H, Steger M, Bleier L, Wumaier Z *et al.* Assembly and oligomerization of human ATP synthase lacking mitochondrial subunits a and A6L. *Biochim Biophys Acta* 2010; **1797**: 1004–1011.
147 Rubinstein J, Walker J. ATP synthase from Saccharomyces cerevisiae: location of the OSCP subunit in the peripheral stalk region. *J Mol Biol* 2002; **321**: 613–619.
148 Mayr JA, Havlickova V, Zimmermann F, Magler I, Kaplanova V, Jesina P *et al.* Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. *Hum Mol Genet* 2010; **19**: 3430–3439.
149 Arnold I, Pfeiffer K, Neupert W, Stuart RA, Schagger H. Yeast mitochondrial F1F0-ATP synthase exists as a dimer: identification of three dimer-specific subunits. *EMBO J* 1998; **17**: 7170–7178.
150 Habersetzer J, Ziani W, Larrieu I, Stines-Chaumeil C, Giraud MF, Brethes D *et al.* ATP synthase oligomerization: from the enzyme models to the mitochondrial morphology. *Int J Biochem Cell Biol* 2013; **45**: 99–105.
151 Wittig I, Velours J, Stuart R, Schagger H. Characterization of domain interfaces in monomeric and dimeric ATP synthase. *Mol Cell Proteomics* 2008; **7**: 995–1004.
152 Strauss M, Hofhaus G, Schroder RR, Kuhlbrandt W. Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *EMBO J* 2008; **27**: 1154–1160.
153 Davies KM, Anselmi C, Wittig I, Faraldo-Gomez JD, Kuhlbrandt W. Structure of the yeast F1Fo-ATP synthase dimer and its role in shaping the mitochondrial cristae. *Proc Natl Acad Sci USA* 2012; **109**: 13602–13607.
154 Dienhart M, Pfeiffer K, Schagger H, Stuart RA. Formation of the yeast F1F0-ATP synthase dimeric complex does not require the ATPase inhibitor protein, Inh1. *J Biol Chem* 2002; **277**: 39289–39295.
155 Arselin G, Giraud MF, Dautant A, Vaillier J, Brethes D, Coulary-Salin B *et al.* The GxxxG motif of the transmembrane domain of subunit e is involved in the dimerization/oligomerization of the yeast ATP synthase complex in the mitochondrial membrane. *Eur J Biochem* 2003; **270**: 1875–1884.
156 Baker LA, Watt IN, Runswick MJ, Walker JE, Rubinstein JL. Arrangement of subunits in intact mammalian mitochondrial ATP synthase determined by cryo-EM. *Proc Natl Acad Sci USA* 2012; **109**: 11675–11680.
157 Minauro-Sanmiguel F, Wilkens S, Garcia JJ. Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis. *Proc Natl Acad Sci USA* 2005; **102**: 12356–12358.
158 Daum B, Walter A, Horst A, Osiewacz HD, Kuhlbrandt W. Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria. *Proc Natl Acad Sci USA* 2013; **110**: 15301–15306.
159 Campanella M, Casswell E, Chong S, Farah Z, Wieckowski MR, Abramov AY *et al.* Regulation of mitochondrial structure and function by the F1Fo-ATPase inhibitor protein, IF1. *Cell Metab* 2008; **8**: 13–25.
160 Campanella M, Parker N, Tan CH, Hall AM, Duchen MR. IF(1): setting the pace of the F(1)F(o)-ATP synthase. *Trends Biochem Sci* 2009; **34**: 343–350.
161 Garcia JJ, Morales-Rios E, Cortes-Hernandez P, Rodriguez-Zavala JS. The inhibitor protein (IF1) promotes dimerization of the mitochondrial F1F0-ATP synthase. *Biochemistry* 2006; **45**: 12695–12703.
162 Feniouk BA, Yoshida M. Regulatory mechanisms of proton-translocating F(O)F (1)-ATP synthase. *Results Probl Cell Differ* 2008; **45**: 279–308.
163 Gledhill JR, Montgomery MG, Leslie AG, Walker JE. How the regulatory protein, IF (1), inhibits F(1)-ATPase from bovine mitochondria. *Proc Natl Acad Sci USA* 2007; **104**: 15671–15676.
164 Faccenda D, Campanella M. Molecular regulation of the mitochondrial F(1)F(o)-ATPsynthase: physiological and pathological significance of the inhibitory factor 1 (IF(1)). *Int J Cell Biol* 2012; **2012**: 367934.
165 Metelkin E, Demin O, Kovacs Z, Chinopoulos C. Modeling of ATP-ADP steady-state exchange rate mediated by the adenine nucleotide translocase in isolated mitochondria. *FEBS J* 2009; **276**: 6942–6955.
166 Chinopoulos C, Gerencser AA, Mandi M, Mathe K, Torocsik B, Doczi J *et al.* Forward operation of adenine nucleotide translocase during F0F1-ATPase reversal: critical role of matrix substrate-level phosphorylation. *FASEB J* 2010; **24**: 2405–2416.
167 Chinopoulos C. Mitochondrial consumption of cytosolic ATP: not so fast. *FEBS Lett* 2011; **585**: 1255–1259.
168 Chinopoulos C. The 'B space' of mitochondrial phosphorylation. *J Neurosci Res* 2011; **89**: 1897–1904.
169 Kiss G, Konrad C, Doczi J, Starkov AA, Kawamata H, Manfredi G *et al.* The negative impact of alpha-ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation. *FASEB J* 2013; **27**: 2392–2406.
170 Kiss G, Konrad C, Pour-Ghaz I, Mansour JJ, Nemeth B, Starkov AA *et al.* Mitochondrial diaphorases as NAD+ donors to segments of the citric acid cycle that support substrate-level phosphorylation yielding ATP during respiratory inhibition. *FASEB J* (e-pub ahead of print 3 January 2014; doi:10.1096/fj.13-243030).
171 Faccenda D, Tan CH, Seraphim A, Duchen MR, Campanella M. IF1 limits the apoptotic-signalling cascade by preventing mitochondrial remodelling. *Cell Death Differ* 2013; **20**: 686–697.
172 Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev Cell* 2011; **21**: 92–101.
173 Suen DF, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev* 2008; **22**: 1577–1590.
174 Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ. Role of Bax and Bak in mitochondrial morphogenesis. *Nature* 2006; **443**: 658–662.
175 Chinopoulos C, Adam-Vizi V. Modulation of the mitochondrial permeability transition by cyclophilin D: moving closer to F(0)-F(1) ATP synthase? *Mitochondrion* 2012; **12**: 41–45.
176 Minkov IB, Fitin AF, Vasilyeva EA, Vinogradov AD. Mg2+-induced ADP-dependent inhibition of the ATPase activity of beef heart mitochondrial coupling factor F1. *Biochem Biophys Res Commun* 1979; **89**: 1300–1306.
177 Fitin AF, Vasilyeva EA, Vinogradov AD. An inhibitory high affinity binding site for ADP in the oligomycin-sensitive ATPase of beef heart submitochondrial particles. *Biochem Biophys Res Commun* 1979; **86**: 434–439.
178 Roveri OA, Muller JL, Wilms J, Slater EC. The pre-steady state and steady-state kinetics of the ATPase activity of mitochondrial F1. *Biochim Biophys Acta* 1980; **589**: 241–255.
179 Drobinskaya IY, Kozlov IA, Murataliev MB, Vulfson EN. Tightly bound adenosine diphosphate, which inhibits the activity of mitochondrial F1-ATPase, is located at the catalytic site of the enzyme. *FEBS Lett* 1985; **182**: 419–424.
180 Bulygin VV, Vinogradov AD. Interaction of Mg2+ with F0.F1 mitochondrial ATPase as related to its slow active/inactive transition. *Biochem J* 1991; **276**(Pt 1): 149–156.
181 Galkin MA, Vinogradov AD. Energy-dependent transformation of the catalytic activities of the mitochondrial F0 x F1-ATP synthase. *FEBS Lett* 1999; **448**: 123–126.
182 Roos I, Crompton M, Carafoli E. The role of inorganic phosphate in the release of Ca2+ from rat-liver mitochondria. *Eur J Biochem* 1980; **110**: 319–325.
183 Crompton M, Ellinger H, Costi A. Inhibition by cyclosporin A of a Ca2+-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. *Biochem J* 1988; **255**: 357–360.
184 Kalashnikova T, Milgrom YM, Murataliev MB. The effect of inorganic pyrophosphate on the activity and Pi-binding properties of mitochondrial F1-ATPase. *Eur J Biochem* 1988; **177**: 213–218.
185 Moyle J, Mitchell P. Active/inactive state transitions of mitochondrial ATPase molecules influenced by Mg2+, anions and aurovertin. *FEBS Lett* 1975; **56**: 55–61.
186 Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N *et al.* Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. *Oncogene* 2000; **19**: 307–314.
187 Wang SB, Murray CI, Chung HS, Van Eyk JE. Redox regulation of mitochondrial ATP synthase. *Trends Cardiovasc Med* 2013; **23**: 14–18.
188 Bernardi P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *J Biol Chem* 1992; **267**: 8834–8839.
189 Petronilli V, Cola C, Bernardi P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore. II. The minimal requirements for pore induction underscore a key role for transmembrane electrical potential, matrix pH, and matrix Ca2+. *J Biol Chem* 1993; **268**: 1011–1016.
190 Scorrano L, Petronilli V, Bernardi P. On the voltage dependence of the mitochondrial permeability transition pore. A critical appraisal. *J Biol Chem* 1997; **272**: 12295–12299.
191 Wittig I, Schagger H. Structural organization of mitochondrial ATP synthase. *Biochim Biophys Acta* 2008; **1777**: 592–598.
192 Chinopoulos C, Adam-Vizi V. Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. *FEBS J* 2006; **273**: 433–450.
193 Halestrap AP, Woodfield KY, Connern CP. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *J Biol Chem* 1997; **272**: 3346–3354.
194 Ko YH, Delannoy M, Hullihen J, Chiu W, Pedersen PL. Mitochondrial ATP synthasome. Cristae-enriched membranes and a multiwell detergent screening

assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP. *J Biol Chem* 2003; **278**: 12305–12309.
195 Acin-Perez R, Fernandez-Silva P, Peleato ML, Perez-Martos A, Enriquez JA. Respiratory active mitochondrial supercomplexes. *Mol Cell* 2008; **32**: 529–539.
196 Genova ML, Baracca A, Biondi A, Casalena G, Faccioli M, Falasca AI *et al.* Is supercomplex organization of the respiratory chain required for optimal electron transfer activity? *Biochim Biophys Acta* 2008; **1777**: 740–746.
197 Giorgio V, Bisetto E, Soriano ME, Dabbeni-Sala F, Basso E, Petronilli V *et al.* Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex. *J Biol Chem* 2009; **284**: 33982–33988.
198 Chinopoulos C, Konrad C, Kiss G, Metelkin E, Torocsik B, Zhang SF *et al.* Modulation of F0F1-ATP synthase activity by cyclophilin D regulates matrix adenine nucleotide levels. *FEBS J* 2011; **278**: 1112–1125.
199 Alavian KN, Li H, Collis L, Bonanni L, Zeng L, Sacchetti S *et al.* Bcl-xL regulates metabolic efficiency of neurons through interaction with the mitochondrial F1FO ATP synthase. *Nat Cell Biol* 2011; **13**: 1224–1233.
200 Chen YB, Aon MA, Hsu YT, Soane L, Teng X, McCaffery JM *et al.* Bcl-xL regulates mitochondrial energetics by stabilizing the inner membrane potential. *J Cell Biol* 2011; **195**: 263–276.
201 Perciavalle RM, Stewart DP, Koss B, Lynch J, Milasta S, Bathina M *et al.* Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. *Nat Cell Biol* 2012; **14**: 575–583.
202 Symersky J, Osowski D, Walters DE, Mueller DM. Oligomycin frames a common drug-binding site in the ATP synthase. *Proc Natl Acad Sci USA* 2012; **109**: 13961–13965.
203 Shchepina LA, Pletjushkina OY, Avetisyan AV, Bakeeva LE, Fetisova EK, Izyumov DS *et al.* Oligomycin, inhibitor of the F0 part of H+-ATP-synthase, suppresses the TNF-induced apoptosis. *Oncogene* 2002; **21**: 8149–8157.
204 Pucci B, Bertani F, Karpinich NO, Indelicato M, Russo MA, Farber JL *et al.* Detailing the role of Bax translocation, cytochrome c release, and perinuclear clustering of the mitochondria in the killing of HeLa cells by TNF. *J Cell Physiol* 2008; **217**: 442–449.
205 Veenman L, Alten J, Linnemannstons K, Shandalov Y, Zeno S, Lakomek M *et al.* Potential involvement of F0F1-ATP(synth)ase and reactive oxygen species in apoptosis induction by the antineoplastic agent erucylphosphohomocholine in glioblastoma cell lines: a mechanism for induction of apoptosis via the 18 kDa mitochondrial translocator protein. *Apoptosis* 2010; **15**: 753–768.
206 Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M *et al.* Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc Natl Acad Sci USA* 2013; **110**: 5887–5892.
207 Szabadkai G, Chinopoulos C. What makes you can also break you, part II: mitochondrial permeability transition pore formation by dimers of the F1FO ATP-synthase? *Front Oncol* 2013; **3**: 140.
208 Masgras I, Rasola A, Bernardi P. Induction of the permeability transition pore in cells depleted of mitochondrial DNA. *Biochim Biophys Acta* 2012; **1817**: 1860–1866.
209 Greie JC, Heitkamp T, Altendorf K. The transmembrane domain of subunit b of the *Escherichia coli* F1F(O) ATP synthase is sufficient for H(+)-translocating activity together with subunits a and c. *Eur J Biochem* 2004; **271**: 3036–3042.
210 McGeoch JE, Guidotti G. A 0.1-700 Hz current through a voltage-clamped pore: candidate protein for initiator of neural oscillations. *Brain Res* 1997; **766**: 188–194.
211 McGeoch JE, Palmer DN. Ion pores made of mitochondrial ATP synthase subunit c in the neuronal plasma membrane and Batten disease. *Mol Genet Metab* 1999; **66**: 387–392.
212 Azarashvili TS, Tyynela J, Odinokova IV, Grigorjev PA, Baumann M, Evtodienko YV *et al.* Phosphorylation of a peptide related to subunit c of the F0F1-ATPase/ATP synthase and relationship to permeability transition pore opening in mitochondria. *J Bioenerg Biomembr* 2002; **34**: 279–284.
213 Krestinina OV, Grachev DE, Odinokova IV, Reiser G, Evtodienko YV, Azarashvili TS. Effect of peripheral benzodiazepine receptor (PBR/TSPO) ligands on opening of Ca2+-induced pore and phosphorylation of 3.5-kDa polypeptide in rat brain mitochondria. *Biochemistry (Mosc)* 2009; **74**: 421–429.
214 Bonora M, Bononi A, De Marchi E, Giorgi C, Lebiedzinska M, Marchi S *et al.* Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle* 2013; **12**: 674–683.
215 Azarashvili T, Odinokova I, Bakunts A, Ternovsky V, Krestinina O, Tyynela J *et al.* Potential role of subunit c of F0F1-ATPase and subunit c of storage body in the mitochondrial permeability transition. Effect of the phosphorylation status of subunit c on pore opening. *Cell Calcium* 2014; **55**: 69–77.
216 Halestrap AP. What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol* 2009; **46**: 821–831.
217 Galluzzi L, Joza N, Tasdemir E, Maiuri MC, Hengartner M, Abrams JM *et al.* No death without life: vital functions of apoptotic effectors. *Cell Death Differ* 2008; **15**: 1113–1123.
218 Galluzzi L, Kepp O, Trojel-Hansen C, Kroemer G. Non-apoptotic functions of apoptosis-regulatory proteins. *EMBO Rep* 2012; **13**: 322–330.
219 Hao Z, Duncan GS, Chang CC, Elia A, Fang M, Wakeham A *et al.* Specific ablation of the apoptotic functions of cytochrome C reveals a differential requirement for cytochrome C and Apaf-1 in apoptosis. *Cell* 2005; **121**: 579–591.