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Mitochondrial Poly (ADP-ribose) Polymerase: The Wizard of Oz at Work

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Abstract

Among multiple members of the poly(ADP-ribose) polymerase (PARP) family, PARP1 accounts for the majority of PARP activity in mammalian cells. Although PARP1 is predominantly localized to the nucleus, and its nuclear regulatory roles are most commonly studied and are the best characterized, several lines of data demonstrate that PARP1 is also present in the mitochondria, and suggest that mitochondrial PARP (mtPARP) plays an important role in the regulation of various cellular functions in health and disease. The goal of the current article is to review the experimental evidence for the mitochondrial localization of PARP1 and its intra-mitochondrial functions, with focus on cellular bioenergetics, mitochondrial DNA repair and mitochondrial dysfunction. In addition, we also propose a working model for the interaction of mitochondrial and nuclear PARP during oxidant-induced cell death. MtPARP is similar to the Wizard of Oz in the sense that it is enigmatic, it has been elusive for a long time and it remains difficult to be interrogated. mtPARP - at least in some cell types - works incessantly “behind the curtains” as an orchestrator of many important cellular functions.

Keywords

poly(ADP-ribose); PARP; oxidative stress; mitochondria; cell death

PARylation: a common posttranslational protein modification

Poly(ADP-ribosyl)ation (PARylation) is a posttranslational protein modification carried out by poly(ADP-ribose) polymerase (PARP) family. In the course of the reaction, PARPs cleave NAD⁺ and attach ADP-ribose units to various amino acid residues (most commonly arginine, cysteine and lysine) on various acceptor proteins, resulting in the elongation and

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branching of the (ADP-ribose)_n polymer also known as poly(ADP-ribose) (PAR). Not all members of the PARP family possess PARP activity; some catalyze mono(ADP-ribosyl)ation, and the function of some of the more recently identified PARP family members remains to be delineated in detail [1-5]. PARylation is a reversible protein modification; enzymes whose role includes the physiological degradation of PAR include poly(ADP-ribose) glycohydrolase (PARG) and ADP-ribose protein hydrolase-3 (ARH3) [1-5].

Mitochondrial localization of PARP1: direct and indirect evidence

Most of the PARP research to date has focused on the originally identified, principal, nuclear isoform of PARP (PARP1). As far as intramitochondrial PARylation, the field was largely neglected and has not been studied systematically. Moreover, whatever little work has been performed in the field of mitochondrial PARP, it yielded divergent results: some investigators find evidence of PARP enzyme (or PARylation activity) in mitochondria, while others did not. Given the importance of mitochondria in health and disease, and the multitude of mitochondrial proteins PARylation may affect - which, consequently, may affect a wide variety of mitochondrial functions, from electron transport and ATP generation to mitochondrial DNA transactions to cell death processes - it is an important question whether mitochondrial PARP exists and whether intramitochondrial PARylation reactions regulate mitochondrial function in health and disease. In the current article we will summarize the direct and indirect evidence of mitochondrial PARP, and we will attempt to explain the reasons behind the divergent findings, that range from methodological differences in the experimentation to biological differences between different cell-types and tissues.

The milestones of mitochondrial ADP-ribosylation activity in chronological order are shown in Fig. 1. The history of nuclear PARylation goes back to more than 50 years when Chambon's group detected PARP-like enzymatic activity and its PAR product in hen liver nucleus [6]. More than a decade later, Kun and coworkers found a macromolecular enzymatic product of NAD⁺ in rat brain mitochondria, proposing a mitochondrial ADP-ribosylating system with properties similar to diphtheria-toxin-catalyzed ADP-ribosylation of elongation factor 2 (an earlier term for PARP) [7]. Several follow-up studies confirmed extranuclear PARP/PARylation. Roberts and coworkers detected PARP1 in the cytoplasm associated with ribosomes during cell cycle progression of HeLa cells [8]. Burzio discovered enzymatic ADP-ribosyl transferase (ART) activity in mitochondrial extracts of *Xenopus* oocytes, rat liver and testis [9-11]. Interestingly, mitochondrial PARP (mtPARP) in testis sperm had 10 times greater specific enzymatic activity compared to mtPARP in somatic cells [11]. Initially, ADP-ribose units were hypothesized to exist as long-chain oligomer/polymers, but in the early 80's *monomeric* ADP-ribose units were also detected in subcellular fragments of rat hepatic tissues [12,13]. Although high ART activity was detected in intact mitochondria and submitochondrial particles, the "mono(ADP-ribose)-transferase"-like protein was mainly localized in the inner mitochondrial membrane [14-16]. In the 70's-80's, the research of mitochondrial ART activity was hindered by technical limitations (e.g. subcellular contaminations and non-specific enzymatic products during protein isolation processes), which created significant difficulties in the biochemical analysis

of mtPARP. Non-enzymatic ART activity was detected in isolated mitochondrial fractions of beef heart and rat liver after sonication, suggesting the potential contamination of the samples with extra-mitochondrial sources of PAR but the same studies have also detected poly and mono(ADP-ribose)-transferase activity in mitochondria without sonication of intact mitochondria [17]. In retrospect, we suspect that digitonin treatment (which was used to aid the inside-out orientation of the mitochondrial inner membranes) may have been responsible for (at least some of) this non-enzymatic side reaction [18,19].

In the early days of PARP research, significant ADP-ribosyl transferase activity was demonstrated in intact mitochondria and in submitochondrial fractions (outer membrane; innermembrane and mitoplast) [19,20], indicating that ART is a major mitochondrial ADP-ribosyl transferase. Later, mitochondrial ART activity was found to be associated with mitochondrial DNA and was localized to the mitochondrial matrix [21]. This observation was further confirmed by confocal microscopy, demonstrating PARylation in the mitochondrial matrix in HeLa cells [22]. This was another significant milestone in mtPARP research, providing further support for the existence of PARP and PARylation within the mitochondrial compartment. Interestingly, Jorcke and colleagues found that mitochondrial ART activity in bovine liver mitochondria is cysteine-specific [23], in contrast to nuclear PARP1 activities, which were historically considered lysine and arginine-targeted [24]. (Although it must be mentioned that cysteine-specific PARylation has also been reported recently [25].)

Further substantiating the presence and function of mtPARP, Du and colleagues presented evidence for mtPARP and mitochondria-specific PARylation by immunocytochemistry and by Western blot analysis of purified mitochondrial fractions. Both mitochondrial and nuclear PARP immunoreactivity (as well as PARylation catalytic activity) were absent in mitochondrial and nuclear extracts prepared from PARP1^{-/-} fibroblasts (Fig. 2) [26]. In addition, enhanced intra-mitochondrial PARylation, induced by oxidative stress, was reported in cultured cortical neurons and fibroblasts [26]. In spite of all of the above evidence for PARP in mitochondria, many investigators working in the field of PARP remained sceptical with respect to mtPARP [27].

In 2008, using dual-label immuno-electron microscopy Lai and colleagues provided *ex vivo* evidence for the intra-mitochondrial localization of PARP in the rat ipsilateral hippocampus after traumatic brain injury [28]. This observation was further extended by detecting mtPARP *ex vivo* in rat tissues and in HeLa cells [28]. Another study showed enhanced PARylation in isolated rat liver mitochondria upon oxidative and nitrosative stress, independent of nuclear PARP1 activity [29], although this effect appeared to be independent of PARP1 (or mtPARP), and was attributed to mitochondrial alfa-ketoglutarate dehydrogenase activity [29].

Since PARP1 does not have a classical mitochondrial localization signal, it has been suggested that translocation of PARP1 to mitochondria requires an interaction with mitofillin. This interaction, indeed, has been demonstrated by confocal microscopy and Western blot analysis of purified mitochondrial extracts from HeLa cells and fibroblasts [30]. Although mitofillin may support PARP1 translocation to mitochondria by a specific,

alternative pathway [31], the exact mechanism(s) responsible for the translocation of PARP1 into the mitochondria remain to be further investigated.

Several, more recent lines of evidence for mtPARP come from our own laboratory. Módos and colleagues demonstrated that mitochondria isolated from cultured human pulmonary epithelial cells show increased oxygen consumption and enhanced NAD⁺ levels after PARP1 silencing, indirectly supporting the functional role of an intramitochondrial PARP system [32]. Moreover, we have demonstrated intramitochondrial localization of PARP1 in U937 and A549 cells by proximity ligation assay and immunoprecipitation methods [33,34].

Even though the above-listed line of evidence seems substantial, we must point out that several lines of studies were unable to demonstrate extranuclear or mitochondrial PARylation. Yu and colleagues failed to detect PARP1 or PARP activity in the mitochondria of immortalized mouse embryonic fibroblasts upon MNNG and H₂O₂ treatments that induced AIF-release and cell death [35]. Cipriani and colleagues sought to investigate the role of PARP1 in mitochondrial dysfunction in HeLa cells [36]. Upon MNNG treatment, they detected PARP1 in the nuclear, but not in the mitochondrial compartment [36]. Exclusive nuclear localization of PARP1 has been concluded in rat brain, heart, testis, and kidney [37] - in contrast to the findings discussed earlier reporting PARP1 in the mitochondria of testis and brain. Lapucci and colleagues concluded that mitochondrial function is regulated by nuclear PARP (rather than mitochondrial PARP) in SHSY5Y human neuroblastoma cells [38].

Taken together, although several lines of evidence, and multiple experimental techniques (cell fractionation, confocal microscopy, or proximity ligation assay) support the mitochondrial localization of PARP1, the literature remains divided. Differences in cell types or species, different experimental techniques, as well as differences in the stimuli used to induce DNA damage (e.g. MNNG, a DNA damaging agent that primarily acts on nuclear DNA, vs. oxidative stress stimuli, which induce both nuclear and mitochondrial responses), may be responsible for these heterogeneous findings. Nevertheless, after reviewing a substantial body of literature, it is reasonable to conclude that - at least in many cell types - PARP1 does have a significant mitochondrial presence (in addition to its 'normal', more abundant and more ubiquitous nuclear localization), and regulates important cellular events (see below).

Local, intra-mitochondrial PARP-dependent regulatory mechanisms

Mitochondria - to a significant part due to their bacterial origin - maintain many cell-autonomous functions. They can undergo fission and fusion; they replicate their DNA, but in a manner that is independent from nuclear DNA replication and the cell cycle, and, in many pathophysiological conditions, can act as a source of endogenous cell death effectors. Intramitochondrial PARP, via PARylation of various mitochondrial proteins, would be expected to have the capacity to modulate various mitochondrial functions, which, in turn, could have important consequences for the whole cell, both under physiological conditions and in various pathophysiological states. Nuclear PARP1 is well known to play a role in nuclear DNA repair, as well as in the regulation of nuclear/cytoplasmic NAD⁺ pools, but it

is also known to act as a post-translational modulator of a multitude of proteins that are PARylated. Does mitochondrial PARP play similar, local roles in the mitochondrial compartment? Moreover, does mtPARP (under baseline conditions, or perhaps when it becomes activated, or perhaps over-activated) play a role in the maintenance of the physiological mitochondrial functions, or perhaps mtPARP may contribute to certain pathophysiological sequence of events?

First of all, what are the molecular and biochemical characteristics of mtPARP? From the limited number of studies utilizing western blotting techniques, it appears to be a protein of approximately 120 kDa, i.e. similar (or identical) to the molecular weight of “nuclear” PARP1. However, the mtPARP has not yet been purified according to today's experimental standards, and small differences in its structure compared to the nuclear, main form of PARP1 are possible. As far as its biochemical character; mtPARP, similar to PARP1, produces a branched poly(ADP-ribose) polymer; the substructure of this polymer, its half-life and its synthesis and degradation dynamics have not been characterized in detail. According to Burzio and co-workers, (whose work was conducted on rat testis tissue and tissue extracts) the activity of PARP and the number of PARylated proteins is higher in the mitochondrial fraction, compared to the nucleus [11]. The K_m of mtPARP to NAD^+ was found to be 22 μM , while the K_m of nuclear PARP was measured as 210 μM [11]. (It should be mentioned that the 210 μM K_m reported for testis PARP is somewhat higher than the K_m values reported in other experimental systems - which range between 50 and 60 μM [39,40].) Similarly, when nuclear and mitochondrial PARylation activity was compared in fibroblasts, mitochondrial specific PARP activity appeared to be higher than nuclear PARP; both mitochondrial and nuclear PARP activity was reduced by INH2BP and both mitochondrial and nuclear PARP activity was reduced in the absence of nicked DNA (Fig. 3) [26]. The molecular mechanism(s) responsible for this difference remain to be elucidated; several endogenous mechanisms have been reported that can affect the activity of PARP1 (from purines to various sex hormones); some of them work through allosteric mechanisms, others by regulating the binding of substrates to the enzyme's active site [41-43]. It is conceivable that the mitochondria and the nucleus provide different ‘microenvironments’ of the various endogenous PARP-regulatory factors, which contributes to the differential affinity of the mitochondrial vs nuclear PARP to NAD^+ .

If mtPARP has a higher specific activity than nuclear PARP1, one would expect that the PARP-mediated turnover of NAD^+ in the mitochondrial matrix is higher than the turnover of nuclear and cytosolic NAD^+ by PARP1. As discussed elsewhere [44], in most mammalian cells the mitochondrial and the cytosolic NAD^+ pools do not communicate. Indeed, shRNA-mediated silencing of PARP1 has been shown to markedly elevate mitochondrial NAD^+ levels in resting A549 cells (a 4-fold increase) [32], while it did not affect total cellular NAD^+ levels [32]. On the other hand, HEK cells containing an overexpressed mitochondrial PARP (generated by using an expression vector consisting of a truncated PARP1 fused to EGFP and directed to the mitochondrial matrix by an established N-terminal targeting sequence) resulted in a lowering of mitochondrial NAD^+ levels [45]. Thus, mtPARP appears to exert a physiological regulatory effect on matrix NAD^+ levels. Mitochondrial PAR polymers, in turn, are primarily degraded by the catalytic activity of ADP-ribosylhydrolase 3

(ARH3), while the mitochondrial form of PAR glycohydrolase (PARG) - in contrast to its nuclear form - appears to be inactive [45,46].

In addition to the regulation of mitochondrial NAD^+ levels, intramitochondrial PARP activity also results in poly(ADP-ribosyl)ation of various mitochondrial target proteins. The list of mitochondrial proteins that are subject to PARylation is substantial (Table 1): it includes electron transport chain complexes III, carrier proteins, a Krebs cycle protein, DNA repair proteins, transport proteins, transcription-regulating proteins, structural proteins and others [26,28,47-50].

The functional consequences of most of these mitochondrial PARylation reactions have not yet been explored; it is conceivable that the degree of PARylation of mitochondrial proteins affects both specific mitochondrial functions, as well as overall cellular functions, including the regulation of mitochondrial electron transport/ATP generation, the maintenance of mitochondrial DNA integrity, and the regulation of cell death. Although it is not always entirely straightforward to dissect the roles of intramitochondrial PARP and PARylation and the nuclear PARP and PARylation, the current state-of-the-art will be overviewed in the subsequent sections.

Regulation of mitochondrial bioenergetics by mtPARP

The mitochondrial electron transport chain, localized in the mitochondrial inner membrane, generates a proton gradient across the inner membrane and thereby maintains the mitochondrial membrane potential. The proton gradient across the membrane is, in term, used ("harvested") by ATP synthase to generate ATP for the cell. Although the fact that PARP regulates total cellular (or cytosolic) NAD^+ pools has been well established for several decades (including studies by Berger in the 70's in the context of genotoxic agents, studies by Cochrane in the 80's in response to hydrogen peroxide, and studies by Szabo in the 90's in response to peroxynitrite) (overviewed in [1]), the specific role of PARP in the regulation of mitochondrial NAD^+ pools has not been studied until recently. MtPARP appears to directly regulate mitochondrial electron transport under normal conditions. Mitochondria isolated from A549 cells with stable silencing of PARP1 (shPARP1) not only presented with substantially higher resting mitochondrial NAD^+ levels (as already discussed above), but they also exhibited a higher metabolic activity than mitochondria isolated from wild-type cells, characterized by higher oxygen consumption rate, as well as a doubling of their respiratory reserve capacity [32]. Likewise, in the studies with overexpression of mtPARP, the cells responded with mitochondrial respiratory deficiency, partially compensated by enhanced glycolysis [45]. The simplest explanation for these results is that mitochondrial NAD^+ , under the control of mtPARP, directly and immediately affects mitochondrial electron transport and cellular respiration, perhaps via influencing the levels of mitochondrial electron donors like NADPH, or perhaps via 'reprogramming' the mitochondria through modulating mitochondrial sirtuin activities. (Nevertheless, with respect to the interpretation of the A549 studies, it must also be pointed out that - since these studies were done in stably silenced cells - an additional mechanism may be that *nuclear* PARP1 regulates the expression and/or activity of various mitochondrial proteins, which, then, manifests in an altered mitochondrial respiratory function.)

Upon oxidative stress conditions, extensive PARylation was detected in mitochondria of rat brain and of fibroblasts, while in PARP1 deficient cells or after pharmacological inhibition of PARP, PARylation was decreased [26]. Both PARP1 deficiency and PARP inhibition increased cellular NAD⁺ and ATP content, mitochondrial membrane potential and improved cell viability [26]. In addition, treatment of isolated mitochondria with peroxynitrite (an endogenous molecule that is known to induce nitrative damage and mitochondrial dysfunction [51,52]) resulted in reduced mitochondrial respiration and mitochondrial uncoupling, which was prevented by a pharmacological PARP inhibitor, as well as by removal of PAR polymers by co-incubating with PARG enzyme [28]. In a subsequent study, oxidative stress of endothelial cells with H₂O₂ lowered mitochondrial ATP production in isolated mitochondria from bEnd.3 cells, while PARP1 silencing or PARP inhibition increased mitochondrial function [32].

Based on the above studies [28] and additional experiments [48], it appears, then, that - in addition to the regulation of mitochondrial function through influencing matrix NAD⁺ levels - activation of mtPARP, followed by poly(ADP-ribosyl)ation of key mitochondrial proteins (e.g. proteins of the electron transport chain), suppresses their catalytic activity, and reduces the rate of mitochondrial electron transport (and/or induces a partial mitochondrial uncoupling) leading to diminished mitochondrial oxygen consumption (Fig. 3).

We have recently demonstrated that the regulation of mtPARP activity is under the influence of the β -adrenergic receptor/cAMP/protein kinase A [PKA] in oxidatively stressed U937 monocytes *in vitro* via a mechanism that involves the modulation of mtPARP activity through phosphorylation [33]. U937 cells subjected to a relatively modest degree of oxidative stress (elicited by H₂O₂ exposure) induced an early (10 minutes after oxidant challenge) mitochondrial DNA (mtDNA) damage, and resulted in the activation of mtPARP. Over the next hours, cells developed a progressive mitochondrial oxidant production, and we have also detected nuclear PARP1 activation (but mtPARP activation preceded nuclear PARP activation by approximately 6 hours). Cells developed a progressive impairment of mitochondrial function, and eventually progressed into a mixed (necrotic/apoptotic) type cell death. MtPARP activation (as well as all of the subsequent events of cell death) were reduced by inhibition of β -adrenoceptors with propranolol, or by inhibiting the signaling processes that are downstream from β receptors (cAMP accumulation and PKA activation) [33]. Importantly, mitochondrial electron transport, oxygen consumption and ATP production were all dependent on mtPARP activity, and they were all regulated by PKA activation in this *in vitro* experimental system [33]. We have also collected evidence for the ability of recombinant PKA to directly phosphorylate recombinant PARP1; this occurred on serines 465 (which is located in the automodification domain) and 782 and 785 (which are located in the catalytic domain [33]). These findings indicate that mtPARP activity is under the regulation of the β -adrenoceptor/cAMP/PKA axis through the regulation of PARP1 phosphorylation, and thereby the modulation of mtPARP activation.

Regulation of mitochondrial DNA repair by mtPARP

One of the best characterized roles of nuclear PARP1 is its role in the regulation of nuclear DNA repair. Although mitochondrial DNA is substantially different than nuclear DNA,

emerging data show that mtPARP plays an important role in mtDNA repair, in a way that shows both similarities and differences with the mechanisms governed by nuclear PARP1.

A single mammalian cell contains up to several thousands of copies of circular 16.5 kb mitochondrial DNA within 80-700 mitochondria [53,54]. Due to its close proximity to the electron transport chain and also due to its unchromatinized nature, the mitochondrial DNA is continuously exposed to endogenous ROS and thus its mutation rate is 20-100-fold higher than that of the nuclear DNA [55,56]. While mitochondria lack some of the DNA repair activities, such as nucleotide excision repair (NER) [57], they possess robust DNA base excision repair/single-strand breaks repair (BER/SSBR) activity for repairing oxidatively damaged DNA [58,59]. Similarly to the nucleus, both sub-pathways of the BER/SSBR were identified and characterized in mitochondria, namely, single-nucleotide BER [60] and long-patch BER [61-63]. Only two mitochondria-specific BER enzymes were identified so far: DNA polymerase gamma (Pol γ) [64-66] and EXOG (endo/exonuclease (5'-3')), endonuclease G-like) [67-70]. The rest of mitochondrial DNA repair enzymes are usually splice variants of the nuclear isoforms.

Elucidation of role of PARP1 in the maintenance of mitochondrial DNA integrity has not been attempted until fairly recently, due to several technical difficulties. These include methodological difficulties in the quantification of mitochondrial DNA damage, limited accuracy in determination of mitochondrial localization of proteins (association with mitochondrial outer membrane, inner membrane space, mitochondrial matrix) and the limited number of exclusively mitochondrial DNA repair enzymes. Nonetheless, increased (ADP-ribosyl)ation was detected in the mitochondrial fraction of potassium-cyanide treated cells, suggesting a potential link between PARP1 and mtDNA [71]. The first set of evidence for the role of PARP1 in the repair of the mitochondrial DNA came from studies investigating the efficiency of mitochondrial DNA repair following damage induced by a DNA methylating agent (methylnitrosourea) [72]. The authors suggested a role for PARP1 in the maintenance of mitochondrial DNA integrity. However, they also observed a lower number of mutagenic DNA lesions in the mitochondrial DNA of cells derived from PARP1^{-/-} cells as compared to their PARP1^{+/+} counterparts, which led them to conclude that PARP1 may exert a negative effect on the repair of the mitochondrial DNA in resting conditions [72]. Rossi and colleagues, working on mtPARP in human fibroblasts and HeLa cells showed that PARP1 was in complex with DNA ligase 3 (LigIII) and mitochondrial DNA [30] and suggested that PARP1 may be an important member of the mitochondrial DNA repair machinery. Recently, we have provided a comprehensive analysis of the role of PARP1 in mitochondrial DNA repair [34]. We were able to show binary interaction between PARP1 and both mitochondria-specific DNA repair enzymes, namely Pol and EXOG, conforming its mitochondrial localization and as a member of mitochondrial DNA repair complex. Interestingly, stable or transient depletion of PARP1 from cultured lung adenocarcinoma cells (A549) showed faster recovery of mitochondrial DNA integrity after initial oxidative insult, indicating that mtPARP *negatively affects repair of the mitochondrial DNA* [34]. We speculated that this is due to extensive PARylation of mitochondrial DNA repair enzymes, particularly under oxidative stress condition, which hinders mitochondrial DNA repair proficiency by disruption of a DNA repair proficient complex, which can lead to the dissociation of repair enzymes from the mitochondrial DNA [34].

Thus, mtPARP can be viewed as part of the mtDNA repair machinery (EXOG, Pol, LigIII). Moreover, mtPARP is associated to mitochondrial transcription factor A (TFAM) [34]. Since mtPARP has also been shown to be linked to mitofillin [30], one potential working hypothesis may be that mtPARP is stored in the inner membrane by mitofillin during normal physiological conditions, but during oxidative damage, mtPARP may be released into mitochondrial matrix. In turn, mtPARP in the matrix may PARylate various members of the mtDNA repair machinery, thereby inhibiting their activities, culminating in a reduced efficiency of mtDNA repair (Fig. 4).

Whether mitochondrial DNA damage, as a result of mtPARP activation, contributes to oxidative cell death, remains to be investigated in future studies. Since mtDNA encodes several key proteins in the mitochondrial electron transport chain, it is conceivable that the normal replacement of these proteins may become impaired, which, then, may cause mitochondrial ROS production and mitochondrial uncoupling, reduced mitochondrial efficacy, and various subsequent cellular disturbances.

Modulation of mitochondrial cell death signaling by mtPARP

Exposure of mitochondria to various reactive oxygen and nitrogen species leads to mtDNA damage. DNA strand breaks appear to be important triggers for the activation of mtPARP. MtPARP can, then, modify a series of mitochondrial proteins by PARylation and regulate their functions. Some of the earliest known markers of mitochondrial dysfunction are the following: (a) enhanced Ca^{2+} uptake via the mitochondrial permeability transition pore (mPTP), (b) increased mitochondrial ROS production, and (c) decreased mitochondrial membrane potential [73,74].

As already mentioned previously, when mitochondria are exposed to oxidative or nitritive stress, inhibition or genetic deficiency of PARP affords a significant preservation of mitochondrial function [26,28]. Likewise, in whole cells challenged with reactive oxygen or nitrogen species, the inhibition or genetic deficiency of PARP protects from the development of mitochondrial dysfunction, preserves mitochondrial membrane potential, maintains NAD^+ content, and prevents the suppression of cellular respiration [26,32,75]. The preservation of mitochondrial function is also associated with a reduced release of mitochondrial derived cell death effectors, such as cytochrome c and apoptosis-inducing factor (AIF) [26,75]. This effect, in turn, leads to decreased DNA fragmentation, and diminished cell death in various cell types [26,28,75]. These data suggest that mitochondrial events (regulated, at least in part, by mtPARP) contribute to the initiation of the cell death process. The mitochondrial events that regulate the release of mitochondrial cell death effectors may relate to the regulation of various subunits of electron transport chain through PARylation [28], and/or PARylation of Grp75 (mtHsp70) and Hsp60 [28], chaperons with known roles in the regulation of mitochondrial stress response signaling. (For instance, Hsp70 was found to antagonize AIF translocation from mitochondria to nucleus and inhibit AIF function [76,77]. Furthermore, Grp75 interacts with VDAC (voltage-dependent anion channel; a member of permeability transition pore in outer mitochondrial membrane) and IP3R (inositol triphosphate receptor Ca^{2+} release channels) suggesting an important role in the maintenance of Ca^{2+} homeostasis [78]). The emergence of a secondary, mitochondrial

oxidant production (which develops in response to a primary, exogenous oxidative stress stimulus) has also been found to be PARP-dependent and inhibitable by PARP inhibitors or PARP deficiency [55,79].

It should be noted that the temporal relationship between PTP opening and PARP activation has not yet been fully characterized and it may show cell-dependent differences as well as differences based on the type of cellular injury. For example, in oxidative stress-induced necrotic cell death of thymocytes, activation of PARP precedes mitochondrial depolarization and secondary ROS production, and PARP inhibition and PARP deficiency suppresses PTP opening [75]. Also, during parthanatos, PAR polymer has been shown to cause depolarization of mitochondrial membrane potential and opening of the PTP early after injury so that the PAR polymer is now considered as an endogenous mitochondrial toxin [79,80]. In addition to PAR, additional cellular events connecting PARP activation to mitochondrial depolarization can involve intracellular NAD⁺ and ATP depletion as well as RIP1-TRAF2-JNK signaling [79,80]. However, it must also be pointed out that some models (e.g. myocardial reperfusion injury) PARP inhibition/knockout failed to prevent the initial mPTP-mediated depolarization or loss of ATP, but PARP ablation it allowed mitochondrial recovery by 4 hours of reperfusion [81]. Overall, the conclusion is that the temporal and causal relationship between PARP activation and opening of PTP (or, wgen considered broadly, mitochondrial dysfunction) may display cell type and injury-dependent differences.

Although the available information is fragmented and often incomplete (as well as cell-type and stimulus-dependent), the published data, when considered as a whole, suggest that mtPARP, in many (but perhaps not all) pathophysiological conditions may act as an important, early-stage regulator of the cell's mitochondrial response to oxidative stress, and suppression of this response can prevent the subsequent cascade of cell death via multiple mechanisms (including prevention of the progressive, secondary mitochondrial oxidant production, maintenance of mitochondrial NAD⁺ homeostasis, maintenance of mitochondrial electron transport, prevention of mitochondrial uncoupling, inhibition of the PARylation of various mitochondrial proteins, and prevention of the opening of PTP), all of which serve to prevent the release of the various mitochondrial cell death effectors (such as cytochrome c and AIF) from the mitochondria (Fig. 5).

Nuclear/mitochondrial PARP interactions in the control of cell death

A distinct paradigm of PARP-mediated cell death relates to the over-activation of nuclear PARP in response to oxidative stress, followed by cellular energetic catastrophe. This model postulates that over-activation of nuclear PARP1 (for instance, as a result of oxidative and nitrosative stress), leads to massive PARylation of nuclear proteins, which, in turn, depletes cytoplasmatic NAD⁺ pools, with the resulting futile NAD⁺ regeneration impairing cellular energetic reserves and exhausting mitochondrial functions. The above paradigm has been put forward first in the context of genotoxic DNA damage of cancer cells [82], then oxidative damage of leukocytes [83], then nitric oxide-related pancreatic and neuronal injury [84,85], and then peroxynitrite-mediated cell injury [75,86,87]. Later, the importance of this mechanism has been extended into a variety of *in vivo* conditions from shock to reperfusion injury, inflammation and neurodegeneration [reviewed in 1,2,5,88,89].

Dawson's laboratory expanded the nuclear PARP activation paradigm, introducing the concept that nuclear PARP activation communicates to the mitochondria via PAR polymers, which signal to the mitochondria to induce the release of AIF, which, in turn, acts back on the nuclear DNA to cause its fragmentation, contributing to irreversible cell death. This paradigm of PARP mediated cell death, designated as 'parthanatos' (Fig. 6), which has been mostly studied in the context of neuronal injury, is overviewed, in detail, in specialized review articles; the importance of this subject includes the pathomechanisms of acute neuroinjury (stroke, traumatic brain injury) to various forms of neurodegeneration (including Alzheimer's disease and Parkinson's disease). [reviewed in 90-99]. A significant debate remains open in the field of PARylation with respect to the relative contribution of cytosolic NAD⁺ depletion vs. the PARylation of various target proteins vs. the production and action of free PAR polymers to the process of cell death triggered by nuclear PARP1 activation.

What must be emphasized is, however, that in studies discussed above in the current subsection, mitochondria were mainly considered as 'victims'; intramitochondrial PARP has not been invoked, nor has it been considered that nuclear and intramitochondrial processes could work in tandem. We have previously taken the position that the role of PARP1 in cell death and diseases is complex and depends on the cell types, mitochondrial content, the intensity, and the time of oxidative stress [1] and now we extend this notion to also propose that the role of mitochondrial vs. nuclear PARP activation is also dependent on the cell type, the stimulus, the stage of the cell death process, and probably many additional factors and circumstances, culminating in a wide spectrum of different cell death types: necrosis, apoptosis, necroptosis or parthanathos. We also hypothesize that a positive feedback cycle, involving both the nuclear and mitochondrial PARPs contributes to oxidant-induced cell death (Fig. 7). According to our current working hypothesis, mtPARP activation, in response to the initial, exogenous oxidative/nitrative insult, induces a secondary, early-onset, progressive, mitochondrial ROS production, and it may also impair mitochondrial membrane potential. In other words, we consider mtPARP as an early intracellular initiator of the cell death cascade, the earliest "traitor" in the imminent "grand mutiny". Nuclear PARP1 activation, on the other hand, may develop its full force only later on, but when it does, it may take over as the main executioner of cell death. Nuclear PARP1-derived PAR polymers may, then, induce further impairment of mitochondrial function, and the AIF and cytochrome release from the mitochondria, when it attacks the nucleus, may, in turn, induce further nuclear PARP1 activation, thereby further enhancing the vicious cycle.

One of the weaknesses of the field of mtPARP is that, currently, not all the tools are available to dissect the roles of mitochondrial vs. nuclear PARP in health or disease. For example, neither a nuclear-specific, nor a mitochondrial-specific PARP inhibitor exists; similarly, PARP1 deficiency or PARP1 silencing decreases both mitochondrial and nuclear PARP pools. Clearly, this is an area where significant further work (including the development of new experimental tools) is needed; the working hypothesis shown in Fig 7. represents our current understanding of the field, involving both mitochondrial and nuclear processes, as well as their interactions.

The Wizard of Oz: origins, roles, intentions

In the tale, Dorothy has experienced many enigmatic actions of the Wizard, but, when she was able to peek behind the curtain and met him face to face, she was disappointed to see that the Wizard is nothing but a man. We 'PARPologists' have also witnessed many enigmatic actions of mtPARP - from the regulation of cellular energetics, and the regulation of cell death to the regulation of mitochondrial DNA repair. Will it be disappointing if it turns out that mtPARP is nothing but a mitochondrial version of the same old "nuclear" PARP1 (albeit placed in a different location in the cell)?

What is the mode of intracellular transport of PARP1 from the cytoplasm (where it is produced on the ribosomes, just like any other protein) to the mitochondria? One possible explanation might be that mutation in the nuclear localization signal of PARP results in extranuclear accumulation of the protein in HeLa cells [100]. Furthermore, the structure of PARP1 may also be altered by posttranscriptional modifications, resulting in mitochondrial localization. Another, more prosaic possibility may be the mitochondrial translocations of PARP1 without classical mitochondrial localization signal, as is the case for the majority of mitochondrially localized proteins [101].

One emerging area of work with respect to PARP's mitochondrial roles is related to the interactions of PARP and PARylation reactions with sirtuins. Sirtuins are the mammalian homolog of Sir2, a NAD⁺-dependent histone deacetylase that was originally discovered as a mediator of the process of aging in lower species. Seven members (Sirt1–Sirt7) are known; one of them (Sirt3) has mitochondrial localization [102,103]. Since both PARP and sirtuins play key roles in various biological processes (including cellular bioenergetics, signal transduction and cell death) and since PARP and sirtuins share their substrate, NAD⁺, interactions between the two systems may potentially affect multiple cellular processes. There are now several sets of studies investigating the interactions of sirtuins and PARP, both in health and disease; the field has been recently reviewed by several expert groups, with the overarching conclusion being that SIRT1 and PARP-1 mutually inhibit each other's activity, which, in turn, has marked effects on mitochondrial function and cellular energetic homeostasis [98,104,105]. With respect to the interactions of PARP and Sirt3, Alano's group have demonstrated that in NMDA-treated neurons (where cytosolic, but not mitochondrial NAD⁺ levels are depleted), mitochondrial SIRT3 overexpression confers neuroprotective effects [106]. One of the targets of Sirt3 is aldehyde dehydrogenase 2, where PARP and Sirt3 actions can converge in the regulation of oxidative stress induced cell death [107]. In retinal capillary endothelial cells exposed to high concentration of extracellular glucose, Sirt3 expression was suppressed, which, in turn, resulted in a reduction in MnSOD activity through activation of PARP1 [108] (although, whether mitochondrial, or nuclear form has not yet been elucidated). In the same study, overexpression of Sirt3 exerted cytoprotective effects through deacetylation and activation of MnSOD [108]. Although the currently available information is fragmented, based on the findings presented above, it is conceivable that multiple layers of interplay exist between mtPARP and Sirt3.

What, then, are the plans and motivations of the Wizard? Is he 'good', or is he 'bad'? Currently, the majority of the data suggest that selective inhibition of mtPARP may provide

some therapeutic benefits under certain conditions; inhibition of mtPARP boosts mitochondrial energetics, protects cells from oxidative death, and - in stark contrast to nuclear PARP1, whose inhibition tends to hinder nuclear DNA repair - depletion of mtPARP even appears to *improve* mitochondrial DNA integrity. Thus, while nuclear PARP is a therapeutic target in situations when one seeks to disrupt DNA repair (i.e. the approved anti-cancer drug olaparib, an inducer of synthetic lethality), mtPARP may be a potential therapeutic target in situations when one seeks to maintain mitochondrial function (for instance, in various forms of critical illness, in neurotrauma and neurodegeneration). A common question, with respect to the PARP-mediated suicide theory (irrespective whether nuclear or mitochondrial PARP is involved) is this: why would evolution not select out such a dangerous, deleterious protein, which catalyzes the demise of the cell? Multiple authors have attempted to address this question in multiple different ways [1-5,88-99]. One potential answer is that our cells are not “designed” to withhold the type of inflammatory or oxidative damage that is associated with insults like stroke, or myocardial infarction, or Alzheimer's disease. In such cases, when viewed from an individual cell's standpoint, perhaps it is the most “prudent” path for the cell is to die and make sure that a damaged cell does not replicate. However, when an insult develops on a larger scale, and affects multiple cells within the same organ (e.g. the ‘necrotic zone’ during heart attacks), then therapeutic PARP inhibition may be able to preserve some - at least partially viable - tissue, which may sustain the function of the affected organ.

Since mtPARP and nuclear PARP1 are closely related (if not identical), selective inhibition of mtPARP will be difficult to achieve. Nevertheless, the journey must continue; the Wizard is quite versatile (Table 2) and we suspect he has many more tricks up his sleeves.

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Abbreviations

AIF	apoptosis-inducing factor
ARH3	ADP-ribosyl protein hydrolase-3
ART	ADP-ribosyl transferase
BER	base excision repair

EXO	endo/exonuclease (5'-3'), endonuclease G-like
grp75	75 kDa glucose regulated protein
ligIII	DNA ligase 3 MOMP, mitochondrial outer membrane permeabilization
MPT	mitochondrial permeability transition pore
mtDNA	mitochondrial DNA
mtPARP	mitochondrial poly(ADP-ribose) polymerase
mPTP	mitochondrial permeability transition pore complex
PAR	poly(ADP-ribose)
PARP	poly(ADPribose) polymerase
PARG	poly(ADP-ribose) glycohydrolase
PKA	protein kinase A
RNS	reactive nitrogen species
ROS	reactive oxygen species
SSBR	single-strand break repair
TFAM	mitochondrial transcription factor A
VDAC	voltage-dependent anion channel

Highlights

- Although PARP1 is predominantly localized to the nucleus, and its nuclear regulatory roles are most commonly studied and are the best characterized, several lines of data demonstrate that PARP1 is also present in the mitochondria, and suggest that mitochondrial PARP (mtPARP) plays an important role in the regulation of various cellular functions in health and disease.
- The current article reviews the experimental evidence for the mitochondrial localization of PARP1 and its intra-mitochondrial functions, with focus on cellular bioenergetics, mitochondrial DNA repair and mitochondrial dysfunction. In addition, we also propose a working model for the interaction of mitochondrial and nuclear PARP during oxidant-induced cell death.

Fig. 1. Milestones in mtPARP research

Fig. 2. PARP-1 and poly(ADP-ribosylation) in mitochondria

a, PARP1 was detected in both nuclear and mitochondrial fractions from PARP1^{+/+} but not PARP1^{-/-} cells (representative of 5 wells/group). **b**, PARP activity in nuclear and mitochondrial protein lysates obtained from PARP1^{+/+} fibroblasts. A lesser degree of PARP activity was detected in nuclear and mitochondrial protein lysates obtained from PARP1^{-/-} fibroblasts (n = 3/group; cpm, counts per min). **c**, PARP activity in nuclear and mitochondrial protein lysates obtained from naive adult rat brain is inhibited by INH₂BP in a dose-dependent manner (-DNA, incubated without exogenous nicked DNA; mean ± S.D.; n = 4 samples/group; *p<0.05 versus no INH₂BP, one-way analysis of variance with Tukey post-hoc test). **d**, confocal dual-label immunohistochemical images with differential interference contrast using antibodies against poly(ADP-ribose) polymers (red) and the mitochondrial marker Hsp60 (green) detected poly(ADP-ribosylation) in mitochondria under baseline conditions (yellow; arrows). Published by permission [26].

Fig. 3. MtPARP inhibits electron transport chain complexes via PARYlation during the early stages of oxidative stress: a working hypothesis

(A) In physiological conditions, Complexes I-IV of the mitochondrial electron transport chain carry electrons and create an electrochemical gradient by pumping H^+ from the mitochondrial matrix to the intermembrane space. This proton gradient is then harvested by ATP synthase (Complex V) to produce ATP. MtPARP is bound to mitofillin and has low basal catalytic activity. (B) In response to oxidative stress, mtPARP is released from mitofillin into the mitochondrial matrix, becomes catalytically activated, and PARYlates various complexes of the electron transport chain, impairing their activity. In addition, mtPARP activation consumes mitochondrial NAD^+ levels, which may also suppress mitochondrial electron transport. Dysregulation of mitochondrial electron transport may also result in mitochondrial uncoupling, which is known to produce a secondary increase in reactive oxygen species by the mitochondria.

Fig. 4. MtpARP inhibits mitochondrial DNA repair during oxidative stress: a working hypothesis
(A) Under physiological conditions, mtPARP is involved in the repair of the mitochondrial DNA by interacting with EXOG and Pol, essential enzymes of the mitochondrial DNA repair machinery. **(B)** In response to oxidative stress, mtDNA becomes damaged and mtPARP becomes activated. In turn, mtPARP PARylates EXOG and Pol, thereby inducing the disassembly of the mitochondrial repair complex, resulting in the inhibition of mitochondrial DNA repair.

Fig. 5. Role of mtPARP in mitochondrial cell death signaling during oxidative stress: a working hypothesis

In response to oxidative stress, mtDNA becomes damaged and mtPARP dissociates from mitofilin and becomes activated. In turn, it PARylates several constituents of Complexes III, IV and V, which suppresses their activity. This leads to mitochondrial uncoupling and secondary, intramitochondrial reactive oxygen species production on Complex IV. Inhibition of complex V would be expected to attenuate mitochondrial ATP production, contributing to the development of cellular bioenergetic failure. PARylation of VDAC-1 and HSP60 by mtPARP may stabilize mPTP in the open position, leading to the facilitation of $[Ca^{2+}]_m$ efflux, secondary ROS production, and, ultimately, the release of cytochrome C (a principal mitochondrial cell death effector) into the cytoplasm.

Fig. 6. Role of nuclear PARP activation in oxidative stress-mediated cell death: the parthanatos working hypothesis

During oxidative stress, nuclear PARP1 becomes activated and PARylates various acceptor proteins. Subsequently, PAR polymers are released from these PARylated proteins by PARG and other enzymes. The ‘free’ PAR polymers enter the cytoplasm, translocate to the mitochondria (yellow arrow), and facilitate mitochondrial AIF release. In turn, AIF reaches the nucleus, and triggers chromatin condensation and further DNA damage (purple arrow). This process, in turn, may produce additional PAR polymer, further amplifying this vicious cycle (red arrow). Overactivation of PARP1 due to nuclear DNA damage also consumes nuclear and cytosolic NAD^+ pools, and, secondarily, cellular ATP pools, which creates cellular bioenergetic dysfunction and contributes to cell death.

Fig. 7. Proposed model of the interplay of mtPARP and nuclear PARP1 during oxidative stress mediated cell death

During oxidative stress, the processes outlined in Figs. 3-6 may occur simultaneously (although the relative contribution of each component may be dependent on the stimulus of cell injury, the cell type, the species, as well as many other factors). MtPARP primarily plays a role in the early onset (minutes) events after oxidative stress, through the initiation of superoxide production by the mitochondria, as well as the subsequent intracellular generation of ONOO^- . Moreover, mtPARP, activated by strand breaks in the mitochondrial DNA, PARylates mitochondrial proteins, increases $[\text{Ca}^{2+}]_m$, decreases $\Delta\psi_m$, and promotes mitochondrial dysfunction. These processes not only contribute to cell dysfunction, but may also sensitize the mitochondria to subsequent events triggered by nuclear PARP1 activation (see below). In a subsequent stage of the process of cell dysfunction (minutes to hours) PARP1 is activated in the nucleus by superoxide and ONOO^- . Nuclear PARP1 activation depletes cellular NAD^+ content and ATP content. Moreover, PAR translocation to the mitochondria induces AIF-release (further promoting nuclear DNA damage), while mitochondrial outer membrane protein PARylation may further promote mitochondrial dysfunction and the release of mitochondrial 'death signals' (such as AIF and cytochrome c), further exacerbating the cell death process. In summary, the above combination of events results in cell death that incorporates elements of necrosis, as well as apoptosis.

Table 1
Mitochondrial proteins subject to PARylation

Protein	Gene symbol	Function	Reference
ELECTRON TRANSPORT CHAIN COMPLEX CONSTITUENTS:			
Apoptosis-inducing factor 1	AIFM1	NADH oxidoreductase and a regulator of apoptosis	49, 50
ATP synthase F(0) complex subunit B1	ATP5F1	a subunit of mitochondrial ATP synthase	28, 49, 50
ATP synthase subunit alpha	ATP5A1	a subunit of mitochondrial ATP synthase	28, 49, 50
ATP synthase subunit beta	ATP5B	a subunit of mitochondrial ATP synthase	28, 49, 50
ATP synthase subunit d	ATP5H	a subunit of mitochondrial ATP synthase	49, 50
ATP synthase subunit gamma, mitochondrial	ATP5C1	a subunit of mitochondrial ATP synthase	28, 49, 50
ATP synthase subunit O	ATP5O	a subunit of mitochondrial ATP synthase	28, 49, 50
Complex I intermediate-associated protein 30	NDUFAF1	complex I assembly factor protein	49, 50
Cytochrome b-c1 complex subunit 1	UQCRC1	part of the ubiquinol-cytochrome c reductase complex	49, 50
Cytochrome b-c1 complex subunit 2	UQCRC2	part of the ubiquinol-cytochrome c reductase complex	28, 49, 50
Cytochrome b-c1 complex subunit Rieske	UQCRFS1	part of the ubiquinol-cytochrome c reductase complex	49, 50
Cytochrome c oxidase subunit 4 isoform 1	COX4I1	part of Cytochrome c oxidase	49, 50
Cytochrome c oxidase subunit 5A	COX5A	part of Cytochrome c oxidase	28, 49, 50
Cytochrome c oxidase subunit 5B	COX5B	part of Cytochrome c oxidase	49, 50
Isoform 2 of Evolutionarily conserved signaling intermediate in Toll pathway	ECSIT	oxidoreductase; required for the efficient assembly of Complex I	49, 50
NAD(P) transhydrogenase	NNT	an integral protein of the inner mitochondrial membrane	49, 50
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10	NDUFA10	part of Complex I 42kDa subunit family	49, 50
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9	NDUFA9	a subunit of the NADH:ubiquinone oxidoreductase, Complex I	49, 50
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5	NDUFB5	a subunit of the NADH:ubiquinone oxidoreductase, Complex I	49, 50
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8	NDUFB8	a subunit of Complex I	49, 50
NADH dehydrogenase [ubiquinone] flavoprotein 1	NDUFV1	51 kDa subunit of the NADH:ubiquinone oxidoreductase Complex I	49, 50
NADH dehydrogenase [ubiquinone] flavoprotein 2	NDUFV2	the 24 kDa subunit of Complex I, electron transfer	49, 50
NADH dehydrogenase [ubiquinone] flavoprotein 3	NDUFV3	part of NADH-ubiquinone oxidoreductase complex I	49, 50
NADH dehydrogenase [ubiquinone] iron-sulfur protein 2	NDUFS2	a core subunit of Complex I	49, 50

Protein	Gene symbol	Function	Reference
NADH dehydrogenase [ubiquinone] iron-sulfur protein 3	NDUFS3	one of the iron-sulfur protein components of Complex I	49, 50
NADH dehydrogenase [ubiquinone] iron-sulfur protein 4	NDUFS4	an nuclear-encoded accessory subunit of Complex I	49, 50
NADH dehydrogenase [ubiquinone] iron-sulfur protein 7	NDUFS7	a subunit of Complex I	49, 50
NADH dehydrogenase [ubiquinone] iron-sulfur protein 8	NDUFS8	a subunit of Complex I	49, 50
Succinate dehydrogenase [ubiquinone] flavoprotein subunit	SDHA	a catalytic subunit of succinate-ubiquinone oxidoreductase, Complex II	49, 50
Succinate dehydrogenase [ubiquinone] iron-sulfur subunit	SDHB	iron-sulfur subunit of succinate-ubiquinone oxidoreductase, Complex II	49, 50
Transmembrane protein 70	TMEM70	Involved in biogenesis of mitochondrial ATP synthase	49, 50
CARRIER PROTEINS:			
Calcium-binding mitochondrial carrier protein Aralar1	SLC25A12	exchange of aspartate for glutamate across the inner mitochondrial membrane	49, 50
Calcium-binding mitochondrial carrier protein Aralar2	SLC25A13	exchange of aspartate for glutamate across the inner mitochondrial membrane	49, 50
Isoform 1 of calcium-binding mitochondrial carrier protein SCaMC-1	SLC25A24	a carrier protein that transports ATP-Mg exchanging it for phosphate	49, 50
Isoform 2 of magnesium transporter MRS2 homolog	MRS2	mitochondrial magnesium channel	49, 50
Isoform B of phosphate carrier protein	SLC25A3	catalyzes the transport of phosphate into the mitochondrial matrix	49, 50
Mitochondrial 2-oxoglutarate/malate carrier protein	SLC25A11	oxoglutarate/malate carrier transports 2-oxoglutarate across the inner membranes of mitochondria	49, 50
Mitochondrial carrier homolog 1	MTCH1	induces apoptosis independent of the proapoptotic proteins Bax and Bak	49, 50
Mitochondrial carrier homolog 2	MTCH2	play a regulatory role in adipocyte differentiation and biology	49, 50
Mitochondrial dicarboxylate carrier	SLC25A10	protein exchanges dicarboxylates, such as malate and succinate, for phosphate, sulfate, and other small molecules	49, 50
Mitochondrial glutamate carrier 1	SLC25A22	a mitochondrial glutamate carrier	49, 50
KREBS-CYCLE PROTEIN:			
Malate dehydrogenase	MDH2	catalyzes the reversible oxidation of malate to oxaloacetate	49, 50
CHAPERONS:			
10 kDa heat shock protein	HSPE1	a major heat shock protein which functions as a chaperonin	28, 49, 50
60 kDa heat shock protein	HSPD1	essential for the folding and assembly of newly imported proteins in the mitochondria	28, 49, 50

Protein	Gene symbol	Function	Reference
HSPA9 heat shock protein family A (Hsp70) member 9	GRP75	cell proliferation, stress response and maintenance of the mitochondria	28, 49, 50
Mitochondrial chaperone BCS1	BCS1L	involved in the assembly of complex III, no mitochondrial targeting sequence	49, 50
<u>DNA-REPAIR PROTEINS:</u>			
Poly(ADP-ribose) polymerase 1	PARP1	DNA repair, bioenergetics	27, 28, 49, 50
DNA-directed RNA polymerase, mitochondrial precursor	POLRMT	mitochondrial gene expression, initiation of replication of the mitochondrial genome	49, 50
endo/exonuclease (5'-3'), endonuclease G-like	EXOG	catalyzes the hydrolysis of ester linkages at the 5' end of a nucleic acid chain	34, 49, 50
Lon protease homolog	LONP1	regulation of mitochondrial gene expression and in the maintenance of the integrity of the mitochondrial genome	49, 50
mitochondrial DNA topoisomerase I	TOP1MT	catalyzes the transient breaking and rejoining of DNA to relieve tension and DNA supercoiling	49, 50
polymerase (DNA directed), gamma	POLG	the catalytic subunit of mitochondrial DNA polymerase	34, 49, 50
Single-stranded DNA-binding protein	SSBP1	maintenance of mitochondrial genome stability	49, 50
<u>TRANSPORT PROTEINS:</u>			
Mitochondrial import inner membrane translocase subunit Tim17-B	TIMM17B	facilitates the transport of mitochondrial proteins from the cytosol across the mitochondrial inner membrane	49, 50
Mitochondrial import inner membrane translocase subunit Tim23	TIMM23	mediates the transport of transit peptide-containing proteins across the membrane	49, 50
Mitochondrial import inner membrane translocase subunit TIM50	TIMM50	essential component of the TIM23	49, 50
Mitochondrial import receptor subunit TOM20 homolog	TOMM20	recognition and translocation of cytosolically synthesized mitochondrial preproteins	49, 50
Mitochondrial import receptor subunit TOM22 homolog	TOMM22	responsible for the recognition and translocation of cytosolically synthesized mitochondrial preproteins	49, 50
Mitochondrial import receptor subunit TOM40 homolog	TOMM40	channel-forming protein essential for import of protein precursors into mitochondria	49, 50
Mitochondrial import receptor subunit TOM70	TOMM70A	accelerates the import of all mitochondrial precursor proteins	49, 50
Mitochondrial ornithine transporter 1	SLC25A15	ornithine transport across inner mitochondrial membrane, from the cytoplasm to the matrix	49, 50
Mitofilin	IMMT	mitochondrial inner membrane organization; protein import	28, 49, 50
Tricarboxylate transport protein	SLC25A1	citrate-H ⁺ /malate exchange	49, 50
<u>TRANSCRIPTION REGULATING PROTEINS:</u>			

Protein	Gene symbol	Function	Reference
Complement component 1 Q subcomponent-binding protein	C1QBP	regulates inflammation and infection processes, ribosome biogenesis, apoptosis, transcription	49, 50
Isoform 1 of pentatricopeptide repeat-containing protein 3	PTCD3	mitochondrial RNA-binding protein	49, 50
Leucine-rich PPR motif-containing protein	LRPPRC	binds to poly(A) mRNA, regulates the stability of mitochondrially encoded cytochrome c oxidase subunits	49, 50
Peptidyl-tRNA hydrolase 2	PTRH2	Aminoacyl-tRNA hydrolase activity	49, 50
Transcription factor A	TFAM	binds to the mitochondrial light strand promoter and functions in mitochondrial transcription regulation	49, 50
transcription factor B1, mitochondrial	TFB1M	a dimethyltransferase that methylates the conserved stem loop of mitochondrial 12S rRNA	49, 50
<u>STRUCTURAL PROTEINS:</u>			
Acyl-CoA dehydrogenase family member 9	ACAD9	Required for mitochondrial complex I assembly	49, 50
Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	CHCHD3	a large protein complex of the mitochondrial inner membrane; maintains crista junctions, inner membrane architecture, and contact sites to the outer membrane	49, 50
Isoform 1 of LETM1 and EF-hand domain-containing protein 1	LETM1	required for the maintenance of the tubular shape and cristae organization	49, 50
<u>DIVISION REGULATING PROTEINS:</u>			
Mitochondrial fission protein MTP18	MTFP1	Involved in the mitochondrial division probably by regulating membrane fission	49, 50
Mitochondrial transmembrane GTPase FZO-2	MFN1	Essential transmembrane GTPase, which mediates mitochondrial fusion	49, 50
<u>FATTY ACID and LIPID METABOLISM:</u>			
Acetyl-CoA acetyltransferase	ACAT1	regulates ketone body metabolism	49, 50
2,4-dienoyl-CoA reductase	DECR1	auxiliary enzyme of beta-oxidation	49, 50
Trifunctional enzyme subunit alpha	HADHA	catalyzes the last three steps of mitochondrial beta-oxidation of long chain fatty acids	49, 50
Isoform 1 of Acylglycerol kinase	AGK	increases cell growth	49, 50
<u>CATABOLIC PROTEINS:</u>			
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	DLAT	catalyzes the conversion of pyruvate to acetyl coenzyme A	49, 50
Glutamate dehydrogenase 1	GLUD1	a mitochondrial matrix enzyme that catalyzes the oxidative deamination of glutamate to alpha-ketoglutarate and ammonia	49, 50
<u>RIBOSOMAL PROTEINS:</u>			
28S ribosomal protein S	MRPS22, MRPS28, DAP3	structural constituents of mitochondrial ribosomes	49, 50

Protein	Gene symbol	Function	Reference
39S ribosomal protein L	MRPL3/4/9/11/13/15/17-19/22-24/28/37-41/43/44/46-49	structural constituents of mitochondrial ribosomes	49, 50
<u>AMINO ACID METABOLISM:</u>			
Aspartate aminotransferase	GOT2	plays a key role in amino acid metabolism	49, 50
Isoform 1 of carbamoyl-phosphate synthase [ammonia]	CPS1	ammonia elimination	49, 50
<u>POLYOL METABOLISM:</u>			
Isoform 1 of glycerol-3-phosphate dehydrogenase	GPD2	synthesizes glycerone phosphate from sn-glycerol 3-phosphate (anaerobic route)	49, 50
<u>GLYCOLYSIS:</u>			
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	glycolysis, NADH production	28, 47
Isoform 1 of pyruvate dehydrogenase E1 component subunit beta	PDHB	catalyzes the conversion of pyruvate; links the glycolytic pathway to the tricarboxylic cycle	49, 50
<u>OTHER PROTEINS:</u>			
Dihydroorotate dehydrogenase	DHODH	catalyzes the conversion of dihydroorotate to orotate with quinone as electron acceptor	49, 50
Isoform 1 of A-kinase anchor protein 1	AKAP1	binds to protein kinase A and anchors it to the cytoplasmic face of the mitochondrial outer membrane	49, 50
Isoform 1 of mitochondrial Rho GTPase 1	RHOT1	controls the anterograde transport of mitochondria and their subcellular distribution	49, 50
Isoform 1 of mitochondrial Rho GTPase 2	RHOT2	regulates mitochondrial trafficking	49, 50
Isoform 1 of serine/threonine-protein phosphatase PGAM5	PGAM5	regulates programmed necrosis	49, 50
Voltage-dependent anion-selective channel protein 1	VDAC-1	controls the diffusion of small hydrophilic molecules	49, 50

Table 2
Cellular functions regulated by mtPARP

	Protein involvement	Reference
mtDNA integrity	EXOG, Pol γ	34
mtDNA repair	mtDNA D-loop, LigIII	30
Mitochondrial mRNA synthesis	TFAM	30
Bioenergetics	Complexes III, IV and V	28
Bioenergetics, cell death	mtPARP, PKA phosphorylation	32,33
Cell death signaling	Mitofillin	30
Cell death signaling	VDAC-1, Hsp60, Grp75	28