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Minireview

Mitochondrial permeability transition and oxidative stress

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Abstract Mitochondrial permeability transition (MPT) is a non-selective inner membrane permeabilization that may precede necrotic and apoptotic cell death. Although this process has a specific inhibitor, cyclosporin A, little is known about the nature of the proteinaceous pore that results in MPT. Here, we review data indicating that MPT is not a consequence of the opening of a pre-formed pore, but the consequence of oxidative damage to pre-existing membrane proteins. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Mitochondrion; Calcium; Reactive oxygen; Free radical; Thiol; Cell death

1. Introduction

After the discovery that mitochondrial components, including the apoptosis inducing factor and cytochrome *c*, can trigger apoptosis [1–5], attention has concentrated on mechanisms through which mitochondria control cell death. One of the many processes that allow mitochondria to release apoptogenic signal molecules into the cytosol is the occurrence of mitochondrial permeability transition (MPT) [3–5]. MPT is a non-selective permeabilization of the inner mitochondrial membrane typically promoted by the accumulation of excessive quantities of Ca^{2+} ions and stimulated by a variety of compounds or conditions (for a review see [6]). The inner membrane permeabilization caused by MPT results in loss of matrix components, impairment of mitochondrial functionality and substantial swelling of the organelle, with consequent outer membrane rupture and cytochrome *c* release [3,6,7].

Despite extensive research, the exact nature of the membrane alterations that lead to MPT still remains unanswered. Since MPT is a partially reversible process [8] mediated by membrane protein thiol oxidation and inhibited by cyclosporin A [6], it clearly involves membrane proteins. However, no novel inner membrane pore to date has shown MPT characteristics. Instead, MPT is almost certainly caused by a group of modified and assembled inner and outer membrane components [9], including the ADP/ATP translocator, cyclophilin D and, possibly, porin and hexokinase [10–13].

Another uncertainty involving MPT is the mechanism through which normal components of mitochondria that participate in MPT assemble to form a non-selective pore upon excessive accumulation of Ca^{2+}. In this article, we review data that strongly suggest that the link between excessive mitochondrial Ca^{2+} accumulation and MPT is oxidative stress.

2. MPT can be induced by mitochondrial pyridine nucleotide oxidation

Early experiments involving ruthenium red-insensitive mitochondrial Ca^{2+} release (now attributable to MPT) showed that this process could be stimulated by promoting the oxidation of mitochondrial pyridine nucleotides, and inhibited or reversed by $NAD(P)^+$ reduction [14]. Subsequent data confirmed that NAD(P) redox status, manipulated through the use of reductants and oxidants, regulated the onset of MPT [10,15–20]. In addition, oxidation of NADPH was shown to be more closely linked to MPT than that of NADH [21]. These findings were a first indication that MPT was related to the redox state of mitochondria and could be caused by reactive oxygen species (ROS), since NADPH plays a central role in mitochondrial defense against oxidative stress. The reductive effect of NADPH on glutathione (GSH) and thioredoxin (TSH), which are substrates for mitochondrial glutathione peroxidase (GPx) and thioredoxin peroxidase (TPx), maintains the functionality of the main H_2O_2 removal enzymes in the organelle [22,23]. Moreover, in the absence of adequate superoxide ($O_2^{\bullet-}$) removal a further oxidation of NAD(P)H could be caused by aconitase inhibition [24], intensifying mitochondrial oxidative stress [25].

An interesting substantiation of the link between MPT and the NAD(P) redox state is the effect of the membrane potential in this process. As long as intramitochondrial Ca^{2+} stores are maintained high, large decreases in transmembrane electrical potential stimulate MPT [15,17,18,20,26–28]. Increased transmembrane electrical potentials, such as those observed in cells overexpressing the anti-apoptotic protein Bcl-2, prevent MPT [29]. This regulation of MPT is most probably mediated by the effects of membrane potential changes on the NAD(P) redox status, as controlled by the membrane potential-sensitive NADP transhydrogenase [22].

The indirect antioxidant effect of the energy-linked NADP

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Abbreviations: GPx, glutathione peroxidase; GSH, glutathione; MPT, mitochondrial permeability transition; P_i, inorganic phosphate; ROS, reactive oxygen species; TPx, thioredoxin peroxidase; TSH, thioredoxin

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transhydrogenase may be an evolutionary defense against oxidative stress, since high membrane potentials favor ROS generation [30]. High membrane potentials are accompanied by slower respiratory rates, which stimulate ROS generation by increasing O_2 tension in the tissues and favoring longer life spans of highly reactive intermediates of the electron transfer chain such as semiquinone radicals [23,30]. Thus, ideal protection against oxidative stress should be obtained under conditions of mild uncoupling (lowering the membrane potential by $\approx$15 mV), when the reaction catalyzed by the NADP transhydrogenase is still shifted toward generating NADPH, but ROS generation is largely diminished [23,31].

3. MPT is promoted by thiol oxidation

Since NAD(P)H redox status is intimately associated with GSH and mitochondrial protein thiol redox state [32], it is logical to suppose that MPT is related to membrane protein thiol status. Indeed, MPT is prevented by thiol reductants such as dithiothreitol [9,33–35], while thiol oxidants such as diamide, phenylarsine oxide and 4,4′-diisothiocyanato-stilbene-2,2′-disulfonic acid promote MPT [21,36,37]. Thiol oxidants and reductants may regulate MPT both by controlling mitochondrial GSH levels or by direct changes in the redox state of MPT pore thiols. Interestingly, Costantini et al. [38] have shown that two distinct membrane thiol pools regulate the opening of the MPT pore, each with reactivity toward distinct thiol reagents.

Thus, we now know that MPT occurs when thiol groups of inner membrane proteins are oxidized, resulting in conformational changes that, somehow, form a large non-selective pore. Interestingly, thiol cross-linkage seems to be essential for these conformational changes, since only dithiol reagents promote MPT. Indeed, cross-linked inner membrane proteins can be observed after MPT on sodium dodecyl sulfate–polyacrylamide gel electrophoresis of solubilized membrane proteins [8,9,35,39]. The proteins which must be cross-linked to result in MPT have still not been determined, but most certainly involve the ADP/ATP translocator [11,12]. In this regard, Vercesi [10] showed evidence for the participation of the ADP/ATP translocator and membrane protein thiol oxidation in the mechanism of $NADP^+$-stimulated Ca^{2+} release from mitochondria. In addition, a critical cysteine residue (Cys56) of the ADP/ATP translocator is oxidized in isolated mitochondria and cells which underwent MPT [40].

4. MPT is inhibited by antioxidants

Based on the evidence that MPT was stimulated by thiol oxidation and depletion of mitochondrial NADPH, we de-

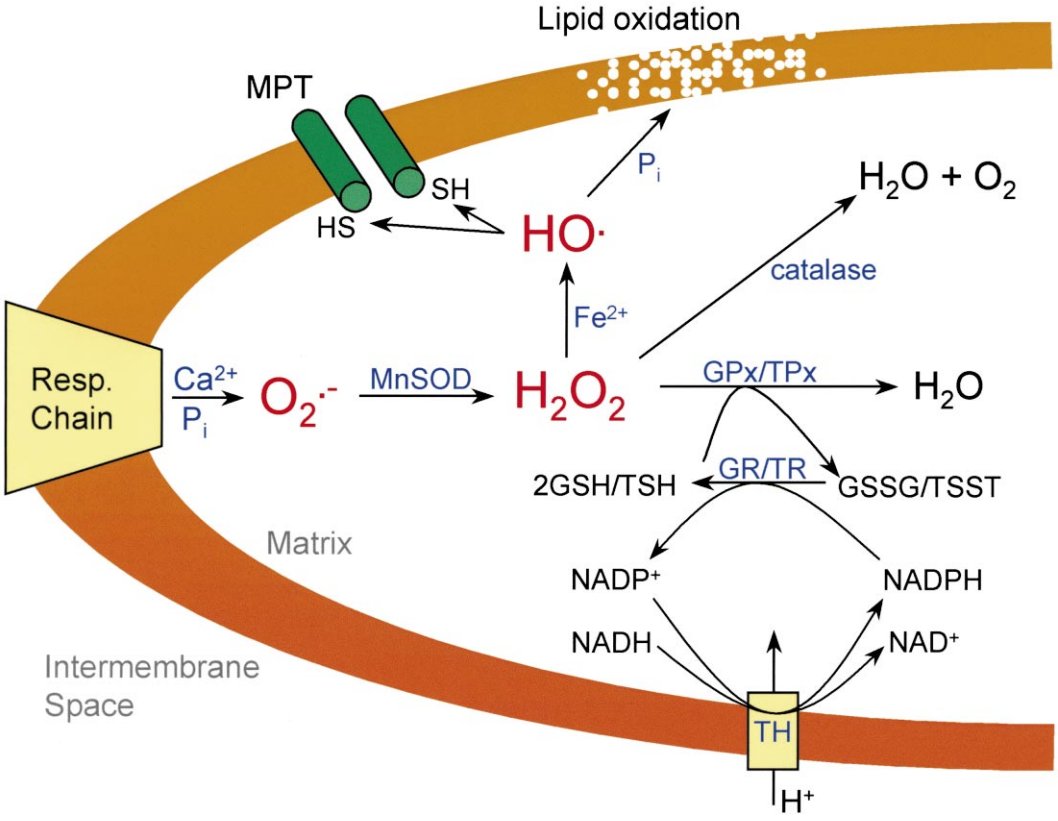

Fig. 1. Mitochondrial ROS accumulation causes MPT. The respiratory chain, inserted in the inner mitochondrial membrane, constantly generates small quantities of superoxide radicals ($O_2^{\bullet -}$). These radicals are normally removed by Mn-superoxide dismutase (MnSOD), which promotes the generation of H_2O_2. H_2O_2 is then reduced to water by GPx, TPx or catalase (in heart mitochondria). GSH, oxidized by GPx, and TSH, oxidized by TPx, are recovered by glutathione and thioredoxin reductases (GR and TR), which use NADPH as an electron donor. NADH, which is available in quantities regulated by respiration and Bcl-2, then reduces $NADP^+$, using the NADP transhydrogenase (TH). When $O_2^{\bullet -}$ generation increases in the presence of Ca^{2+} and P_i, and/or H_2O_2 removal pathways are inactivated, H_2O_2 accumulates in quantities too large for removal, and, in the presence of Fe^{2+}, generates the highly reactive $HO^{\bullet}$ radical. $HO^{\bullet}$ oxidizes thiol (–SH) groups of the MPT pore complex, leading to pore assembly and opening. Alternatively, $HO^{\bullet}$ may also promote membrane permeabilization through lipid oxidation, a process strongly stimulated by P_i.

cided to directly test the hypothesis that MPT was caused by oxidative stress. Confirming our initial proposition, we observed that a wide variety of antioxidants protect against MPT [35,39,41–43]. The finding that catalase and thiol peroxidases protect against MPT was especially significant, since it indicates that MPT can be induced directly by ROS, and depends on the presence of H_2O_2. Mitochondrial H_2O_2 derives from superoxide radicals (see Fig. 1) and is converted to hydroxyl radicals, via the Fe^{2+}-dependent Fenton reaction, before promoting MPT [39].

The obvious source of ROS production leading to MPT is the mitochondrial respiratory chain. We have determined that the semiquinone form of coenzyme Q is a significant generator of superoxide radicals that ultimately promote MPT [44], and that a decrease in the concentration of molecular oxygen in the reaction medium protects against MPT [39]. Although the pronounced inhibition of MPT in anaerobiosis supports the participation of ROS in MPT, it is important to stress that free radicals may be formed through electron donation to components of the mitochondrial suspension even in the absence of oxygen [39]. These free radicals could be responsible for MPT observed under anaerobic conditions [45,46]. However, we have also shown that full removal of molecular oxygen from the mitochondrial suspension is not easily obtained [43], explaining why ROS may be present under apparent anaerobiosis. In the full absence of ROS, MPT appears to be fully inhibited when promoted by Ca^{2+} and inorganic phosphate (P_i) or NAD(P)H oxidants [39,43], but may still be promoted by direct oxidation of inner membrane protein thiols using thiol reagents [21,36,37].

Further evidence that MPT was caused by ROS was provided by the discovery that this process could be promoted through the addition of exogenous sources of ROS [47,48]. Nitrogen-containing ROS such as peroxynitrite cause protein thiol oxidation and MPT, paralleled by membrane lipid oxidation [49,50].

5. Ca^{2+} and P_i lead to mitochondrial oxidative stress

Protection against MPT conferred by antioxidants such as catalase is observed not only when MPT is caused by NAD(P)H oxidation, but also when MPT is induced by Ca^{2+} and P_i in the absence of NAD(P)H oxidants [41–43]. This finding suggests that Ca^{2+} and P_i can, alone, lead to a condition of oxidative stress in mitochondria. Indeed, we measured a large increase in ROS generation when mitochondria were treated with Ca^{2+} and P_i [41–43]. To date, there is no clear understanding why P_i, despite decreasing matrix free Ca^{2+} concentrations, increases ROS generation and stimulates MPT. It is possible that P_i catalyzes reactions that favor ROS formation (see below and [41]).

Grijalba et al. [51] have developed an interesting hypothesis, supported by experimental data, to explain how Ca^{2+} may increase mitochondrial ROS formation. They demonstrated that Ca^{2+} alters the lipid organization of the inner mitochondrial membrane by interacting with the anionic head of cardiolipin, an abundant component of this membrane. These alterations in membrane organization may affect respiratory chain function, including coenzyme Q mobility, and favor monoelectronic oxygen reduction (superoxide radical generation) at intermediate steps of the respiratory chain.

6. MPT can be associated with other forms of mitochondrial oxidative damage

Further evidence for the role of oxidative stress in MPT is the associated occurrence of oxidative damage to non-protein mitochondrial components. Extensive lipid oxidation parallels MPT when mitochondria are treated with high phosphate concentrations [41]. P_i stimulates lipid oxidation due to its ability to catalyze aldehyde tautomerization producing enols, which, when oxidized by heme proteins, yield triplet state aldehydes that are ROS themselves [41,52]. Lipid oxidation is also associated with MPT promoted by peroxynitrate [50]. Lipid oxidation may increase the destructive effects of MPT in cells, since it also promotes mitochondrial swelling, cytochrome *c* release and impaired oxidative phosphorylation. Indeed, the short-term irreversibility of membrane lipid oxidation makes this form of mitochondrial damage potentially more lethal than MPT, which is reversible soon after its onset [8].

7. In situ MPT is related to cellular redox state

While most of our data relating MPT to mitochondrial oxidative stress was collected from isolated organelle studies, a large amount of experiments using intact cells now support our conclusions. Cell death under conditions which may involve MPT can be prevented by antioxidants [53–59], while NAD(P)H oxidants promote mitochondrial oxidative stress and MPT in intact cells [19,60]. In addition, reduced GSH, which prevents MPT, has been shown to promote cell survival after injurious stimuli [58,61–63].

MPT occurring inside cells may be a manner of eliminating individual mitochondria ('mitoptosis') which produce excessive ROS [64]. Thus, MPT may be a last-line cellular defense mechanism against mitochondrially generated ROS.

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