Spotlight

A New Current for the Mitochondrial Permeability Transition

Massimo Bonora[1,*] and Paolo Pinton[1,2,*]

Mitochondrial F1/FO ATP synthase participation in the mitochondrial permeability transition pore complex (PTPC) remains controversial. Neginskaya *et al.* (*Cell Rep.* 2019;26:11–17) reported an unexpected current with PTPC-like properties in F1/FO ATP synthase C subunit knockout cells that could explain part of the conflictual literature.

The mitochondrial permeability transition (MPT) is a thoroughly studied phenomenon of mitochondrial biology, but many aspects remain unexplained. MPT refers to a rapid increase in inner mitochondrial membrane permeability to low molecular weight solutes (<1.5 kDa) that is triggered by Ca^{2+} accumulation in the mitochondrial matrix. The resulting influx of water into the matrix causes structural and functional collapse of the affected mitochondria. The outcome of this detrimental cascade is cell death [1].

The physiology of this phenomenon is very well characterized but its molecular nature remains puzzling. Early electrophysiological recordings established that the MPT is mediated by a regulated channel with a characteristic conductance of ~1.5 nS and an estimated pore size of 2–3 nm [2]. Further investigations involving MPT inhibitors [including cyclosporine A (CsA), ADP, and bongkrekic acid] allowed the identification of many protein components [e.g., voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT), and cyclophilin D (CypD)]. These findings led to the hypothesis that the channel must originate in a supramolecular entity assembled at the interface between the mitochondrial membranes – the so-called PTPC. Nonetheless, robust investigations on knockout (KO) or knockdown (KD) models revealed that most of these proposed components (e.g., ANT) are likely to be regulators of MPT rather than essential constituents [1].

In the past 6 years a new candidate has been proposed as a pore-forming member of the PTPC – the mitochondrial F1/FO ATP synthase (hereafter referred to as ATP synthase). Multiple lines of evidence have established that: (i) genetic manipulation of crucial components of ATP synthase ablates or deeply alters PTPC activity; (ii) isolated ATP synthase, or the key C subunit, can generate Ca^{2+}-induced currents in artificial bilayers; (iii) ATP synthase interacts with CypD (the target of CsA); and (iv) molecules that target the C subunit can impair MPT ([1,3,4] for further reading).

Recently, the ATP synthase model has been questioned by CRISPR/Cas9-mediated KO of C subunits [5]. Indeed, cells depleted in the C subunit still show Ca^{2+}-sensitive and CsA-sensitive alterations of mitochondrial integrity. These studies are undoubtedly valuable because they investigate the MPT in a 'cleaner' ATP synthase-disrupted context. However, careful evaluation of the published results suggested that MPT might be only partially inhibited, and that further investigation of PTPC activity was required.

In a recent issue of *Cell Reports*, Neginskaya and coworkers further characterize MPT in the HAP1-A12 clone that lacks the C subunit [6]. Using electrophysiological recordings of isolated mitoplasts, they observed that these cells have a Ca^{2+}-inducible current that can be inhibited by CsA. This current was slightly different from what is expected for the PTPC (Figure 1). In wild-type (WT) cells the current was ~1.3 nS, but in the KO it was considerably lower at 0.3 nS. This observation implies that the pore formed in the absence of the C subunit is significantly smaller. Thus, the smaller pore size would still allow the flow of small molecules and ions [e.g., Ca^{2+}, Co^{2+}, calcein, tetramethylrhodamine methyl ester (TMRM)], which are commonly used in PTPC characterization, leading to possible misinterpretation of PTPC activity and composition. The authors further investigated this induced current in C subunit KO cells and found that it could be inhibited by ADP and by the ANT inhibitor bongkrekic acid. They concluded that, in C subunit KO cells, a second current may be generated by ANT. This nucleotide transporter has already been described to interact with CypD [7], and this could explain why MPT is also blocked by CsA in the HAP1-A12 clone. Less clear is what makes ANT able to trigger the current in the presence of Ca^{2+}. Nonetheless, such observations again suggest that ANT is a possible mediator of MPT, and this could be verified by knocking down its four isoforms in ATP synthase-deficient cells.

The identification of this second current opens multiple possibilities regarding PTPC channel architecture, but one can speculate about at least two alternatives. First, the two currents are independent and can be Ca^{2+}-triggered or CsA-inhibited concurrently. If so, opening of the C subunit-dependent current in WT cells would mask the effect of the ANT-like current owing to the smaller pore size of the latter. Second, the correct MPT configuration in WT cells may require both ANT and the C subunit. In support of this second hypothesis, the authors observed that mitochondrial depolarization triggered by the MPT-inducer ferutinin is delayed in HAP1 WT cells compared with HAP1-A12 cells that lack the C subunit [6]. These data suggest that complex reorganization of the ATP synthase may be required for channel activity. In support of this finding, we previously reported that ATP synthase converts from a dimeric to a monomeric form during MPT. Furthermore, forcing or destabilizing

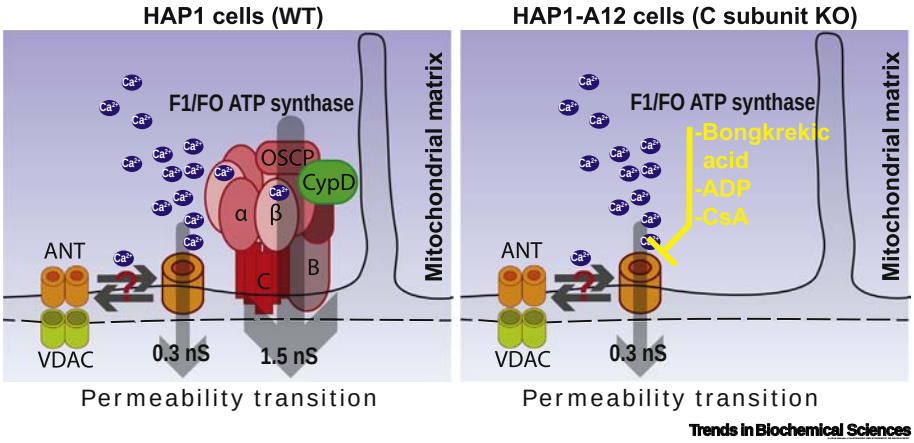

Figure 1. The Mitochondrial Permeability Transition (MPT). During MPT elevated levels of mitochondrial calcium trigger currents of different amplitudes across the inner mitochondrial membrane in wild-type (WT) cells (left panel). The 1.5 nS current was already ascribed to the F1/FO ATP synthase [4], whereas a newly identified 0.3 nS current was proposed to be mediated by adenine nucleotide translocase (ANT) [6]. In C subunit knockout (KO) cells (right panel), the larger current is missing but the smaller is still able to mediate MPT (of moderate amplitude) and shows sensitivity to canonical inhibitors of the MPT complex such as ADP, bongkrekic acid, and cyclosporine A (CsA). Abbreviation: VDAC, voltage-dependent anion channel.

ATP synthase dimer stability by genetic manipulation led to either inhibition or sensitization of the PTPC, respectively [3]. In addition, a previous investigation of ANT1/2 double-KO cells reported that, although MPT was still inducible, it had lost sensitivity to ADP inhibition [8].

The existence of a smaller current might further explain the low- and high-conductance states. The PTPC channel has been reported to exist in two different conformational states with different conductances and sensitivities to inhibitors/inducers, and with different cellular outcomes. More precisely, although the high-conductance state is reported to trigger cell death, the low-conductance state appears to be involved in regulating mitochondrial physiology [9].

Interplay between ANT and ATP synthase and/or their expression levels might thus be relevant for MPT induction and outcome. ANT deficiency indeed allows PTPC opening, although this requires a higher Ca^{2+} concentration. This might lead to switching from a low- to a high-conductance (cytotoxic) state, and thus could contribute to the tissue degeneration observed in many ANT deficiencies [8].

A full understanding of PTPC induction is of strategic importance in the development of therapeutic strategies against pathologic conditions involving MPT. Robust genetic evidence has indeed incriminated MPT as a major etiological determinant in a wide panel of acute and chronic disorders characterized by the unwarranted loss of postmitotic cells. These conditions include ischemia/reperfusion injury of the brain, heart, and kidney; neurodegenerative disorders; toxic syndromes; and myopathic/dystrophic disorders [1].

Several attempts have been launched to pharmacologically inhibit MPT. Unfortunately, no molecule has successfully arrived in clinical practice, at least in part owing to our limited knowledge about the structure and mode of action of the PTPC. The present data open the possibility that there are two distinct routes to MPT. If so, only common regulators (e.g., CypD) might be appropriate targets for drug design. In addition, the contributions of the ANT- and ATP synthase-related currents have not been investigated in all KD combinations or in the same experimental settings. Previous data indicated that ANT1 and 2 KOs do not significantly affect cell death [8]. By contrast, KD of the C subunit does affect cell death, and new classes of molecules able to target the C subunit have now been designed, thus inhibiting MPT without affecting ATP synthesis [10].

In conclusion, this study opens a new perspective on the mechanism of MPT and calls for further investigations to better elucidate the mechanism(s) of pore formation and the regulation of cell death, as well as to allow the development of new drugs for therapeutic applications.

Acknowledgments

We apologize to all authors whose work could not be cited owing to space limitations. P.P. is grateful to Camilla degli Scrovegni for constant support. P.P. is supported by the Italian Association for Cancer Research (AIRC; IG-18624) and Telethon (GGP15219/B); M.B. and P.P. are supported by local funds from the University of Ferrara.

[1]Department of Morphology, Surgery, and Experimental Medicine, Section of Pathology, Oncology, and Experimental Biology, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy
[2]Maria Cecilia Hospital, GVM Care and Research, 48033 Cotignola, Ravenna, Italy

*Correspondence:
bnrmsm1@unife.it (M. Bonora) and
paolo.pinton@unife.it (P. Pinton).

https://doi.org/10.1016/j.tibs.2019.04.009

References
1. Bonora, M. *et al.* (2015) Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition. *Oncogene* 34, 1475–1486
2. Szabo, I. and Zoratti, M. (1992) The mitochondrial megachannel is the permeability transition pore. *J. Bioenerg. Biomembr.* 24, 111–117
3. Bonora, M. *et al.* (2017) Mitochondrial permeability transition involves dissociation of F1FO ATP synthase dimers and C-ring conformation. *EMBO Rep.* 18, 1077–1089
4. Bernardi, P. (2018) Why F-ATP synthase remains a strong candidate as the mitochondrial permeability transition pore. *Front. Physiol.* 9, 1543
5. He, J. *et al.* (2017) Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl. Acad. Sci. U. S. A.* 114, 3409–3414
6. Neginskaya, M.A. *et al.* (2019) ATP synthase c-subunit-deficient mitochondria have a small cyclosporine A-sensitive channel, but lack the permeability transition pore. *Cell Rep.* 26, 11–17
7. Brustovetsky, N. and Klingenberg, M. (1996) Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca^{2+}. *Biochemistry* 35, 8483–8488
8. Kokoszka, J.E. *et al.* (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* 427, 461–465
9. Ichas, F. and Mazat, J.P. (1998) From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochim. Biophys. Acta* 1366, 33–50
10. Morciano, G. *et al.* (2018) Discovery of novel 1,3,8-triazaspiro[4.5]decane derivatives that target the c subunit of F1/FO-adenosine triphosphate (ATP) synthase for the treatment of reperfusion damage in myocardial infarction. *J. Med. Chem.* 61, 7131–7143

Forum

Hutchinson–Gilford Progeria Syndrome: Cardiovascular Pathologies and Potential Therapies

Suowen Xu[1,*] and Zheng-Gen Jin[1]

Hutchinson–Gilford progeria syndrome (HGPS) is an ultrarare and fatal disease with features of premature aging and cardiovascular diseases (atherosclerosis, myocardial infarction, and stroke). Several landmark studies in 2018–2019 have revealed novel mechanisms underlying cardiovascular pathologies in HGPS, and implicate future potential therapies for HGPS, and possibly physiological aging.

Hutchinson–Gilford Progeria Syndrome

Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disease (with an estimated incidence of one in 10 million births) that causes disease phenotypes normally observed in the aged population (e.g., hair loss, skeletal abnormalities, sclerodermatous skin changes, and cardiovascular disease) [1]. HGPS is mainly caused by a *de novo* heterozygous point mutation (c.1824C>T; p.G608G) in the human *LMNA* gene, which generates an alternatively spliced prelamin A transcript that yields a toxic lamin A protein, commonly known as progerin [1]. HGPS is a rare but devastating syndrome affecting multiple organs, including the blood vessel and the cardiac system. Despite its rarity, the last decade has witnessed a tremendous increase in HGPS research (Figure 1A). However, mechanistic links between progerin synthesis and cardiovascular dysfunction in HGPS remain incompletely understood.

Cardiovascular Pathologies of HGPS

Children with HGPS mainly die from cardiovascular disease due to atherosclerosis. It is striking that the children do not have any of the common risk factors for cardiovascular disease (e.g., hyperlipidemia, hypertension, diabetes, and smoking). This suggests that progerin synthesis plays an important pathogenic role in the artery wall [1]. Recently, new mouse models have provided new insights into the mechanisms of how progerin synthesis promotes cardiovascular disease in HGPS. These include mice that express progerin in only endothelial cells (EC) (progerin^{ecTg}) [2]; *ApoE*$^{-/-}$ mice that express progerin in only vascular smooth muscle cells (VSMCs) or macrophages [3]; and *ApoE*$^{-/-}$ mice that express progerin globally (*Lmna*$^{G609G/G609G}$*ApoE*$^{-/-}$) [3]. Atherosclerosis involves the complex interplay of multiple cell types: ECs, VSMCs, monocytes/macrophages, and other immune cells. Understanding how progerin accelerates atherosclerosis development may identify new therapeutic strategies to treat atherosclerosis in HGPS, and possibly the general aging population (Figure 1B).

Endothelial Dysfunction

ECs are the innermost layer of cells lining the blood vessel. They serve as an important interface for substance exchange, vascular homeostasis, and mechanotransduction. To examine whether progerin promotes endothelial dysfunction and cardiovascular pathology in HGPS, Osmanagic-Myers *et al.* [2] generated progerin^{ecTg} mice. Interestingly, they showed early signs of diastolic dysfunction, accompanied by cardiac hypertrophy, perivascular and interstitial fibrosis, and premature death without VSMC loss. Since ECs are constantly exposed to blood flow-induced fluid shear stress, the authors observed that ECs from progerin^{ecTg} mice impaired cellular and cytoskeletal alignment and defective mechanosensitive MRTF-A (myocardin-related transcription factor-A)/eNOS (endothelial nitric oxide synthase) signaling [2]. The accumulation of progerin in ECs makes the nuclear lamina extremely stiff, causing intracellular mechanical stress. This in turn leads to exaggerated fibrosis, vascular stiffness, and cardiac dysfunction [2]. This study provides the first evidence showing that progerin-elicited endothelial dysfunction directly contributes to fibrosis and cardiac impairment in HGPS. However, it remains unclear whether progerin synthesis in ECs is causal in accelerated atherosclerosis in HGPS.

Dysfunction of VSMCs

Depletion of VSMCs in atherosclerotic plaques is closely related to plaque vulnerability and rupture, which are closely linked to mortality rates. VSMC depletion has been observed in mouse models of HGPS and HGPS patients [1]. A recent study performed in *Lmna*^{G609G} knock-in mice [4] showed that progerin promotes VSMC loss and adventitial fibrosis with