Rethinking Mitchell’s Chemiosmotic Theory: Potassium Dominates Over Proton Flux to Drive Mitochondrial F$_{1}$F$_{0}$-ATP Synthase

Edoardo Bertero$^{1,2}$ and Christoph Maack$^{1,3,*}$

$^1$Comprehensive Heart Failure Center (CHFCC), University Clinic Würzburg, Würzburg, Germany, $^2$San Martino Policlinic Hospital, University of Genova, Genova, Italy and $^3$Department of Internal Medicine 1, University Clinic Würzburg, Würzburg, Germany

*Address correspondence to C.M. (e-mail: maack_c@ukw.de).

A Perspective on “ATP synthase K$^+$- and H$^+$-fluxes drive ATP synthesis and enable mitochondrial K$^+$-“uniporter” function: I. Characterization of ion fluxes & “ATP synthase K$^+$- and H$^+$-fluxes drive ATP synthesis and enable mitochondrial K$^+$-“uniporter” function: II. Ion and synthase flux regulation”

Mitochondria are the dominant source of energy in the form of adenosine triphosphate (ATP) in most cells. In the mitochondrial matrix, the Krebs cycle is fueled by nutrients to reduce nicotinamide- (NADH) and flavin adenine dinucleotide (FADH$_2$)$^1$, which donate electrons to the respiratory chain (Figure 1). The ensuing electron transfer along complexes I-IV of the chain and onto oxygen (O$_2$) provides the energy to pump protons (H$^+$) from the matrix to the intermembrane space, generating a chemical (ΔpH) and an electrical potential (ΔΨ$_m$) across the inner mitochondrial membrane (IMM), which together constitute the protonmotive force (Δψ$_m$). According to the chemiosmotic theory developed by Peter D. Mitchell, Δψ$_m$ is the driving force for oxidative phosphorylation of adenosine diphosphate (ADP) to ATP at the F$_{1}$F$_{0}$-ATP synthase (Figure 1)$^1$. This concept, for which Mitchell was awarded the Nobel Prize for Chemistry in 1978, has been accepted for more than 50 years and can be found in literally every textbook of biology.

In the current issue of Function, Juhaszova and colleagues$^3$ substantially challenge—or rather expand, but do not tumble—this concept in revealing that in addition to H$^+$, potassium ion (K$^+$) flux through the F$_{1}$F$_{0}$-ATP synthase (working the same way as H$^+$) provides the majority of energy to produce ATP (Figure 1). Why was this overlooked for more than six decades? Preumably because the F$_{1}$F$_{0}$-ATP synthase has a $> 10^5$-fold selectivity for H$^+$ over other cations.$^4$ But what was not sufficiently considered is that due to the $> 10^5$-fold higher cytosolic concentration for K$^+$ (~100 mM) than for H$^+$ (~100 nM), such that K$^+$ flux—driven mostly by the same high electrical driving force (Δψ$_m$) - could be comparable to H$^+$ flux via the ATP synthase.

Employing a variety of experimental systems, including proteoliposomes containing purified mammalian F$_{1}$F$_{0}$-ATP synthase, planar lipid membranes, but also intact rat cardiac mitochondria, Juhaszova et al.$^3$ elegantly demonstrate that for each H$^+$, 2.7 K$^+$ ions are transferred at the ATP synthase under physiological conditions. Since contraction of intramitochondrial volume hinders the activity of the respiratory chain, and K$^+$ influx osmotically allows water to expand the matrix, such two-ion flux through the F$_{1}$F$_{0}$-ATP synthase not only increases ATP synthesis, but also improves its efficiency: Compared to H$^+$ flux, K$^+$ flux exhibited a 3.5-fold higher ATP synthesis, but only a 2.6-fold higher O$_2$ consumption rate.$^3$ Although a “two-ion theory of energy coupling” was proposed previously by Nath,$^18$ the models differ substantially: while Nath proposed a H$^+$/K$^+$ antiport within the F$_{1}$F$_{0}$-ATP synthase may maintain electroneutrality,$^18$ the model presented here$^3$ defines a H$^+$/K$^+$ symport via the ATP synthase, where K$^+$ extrusion is accounted for by the distinct K$^+$/H$^+$ exchanger (KHE; Figure 1). Importantly, this novel concept, which allows an optimized matching of energy supply to demand, was corroborated by a minimal computational model comprising the “core” mechanism constituted by ATP synthase, driven by both H$^+$- and K$^+$-motive force, respiratory chain, adenine nucleotide translocator, phosphate carrier, and the K$^+$/H$^+$ exchanger in a parallel study published elsewhere.$^5$

As if this discovery was not enough of a scientific earthquake, in a second manuscript, the same authors$^3$ uncover that by this previously unrecognized K$^+$ flux, the F$_{1}$F$_{0}$-ATP synthase
to form a channel with mKATP-like properties when associating with the ATP Binding Cassette protein 8 (ABCB8), which had already been shown to modulate mKATP activity. Since knock-out of CCDC51 in vivo confirmed its essential role to regulate mitochondrial function in unstressed conditions and protect from necrosis during ischemia/reperfusion, CCDC51 and ABCB8 are currently the most accepted candidates in the field to constitute the mKATP (Figure 1).

The second study by Juhaszova et al. in this issue of Function delineates the endogenous and exogenous regulation of the F$_1$F$_\text{ε}$-ATP synthase in its function as a K$_\text{ATP}$ channel. The survival-related protein Inhibitory Factor 1 (IF1) is regulated by Bcl-family proteins, in particular Bcl-xl and Mcl-1, but not Bcl-2, through interaction at a BH3-like domain, which increases chemo-mechanical efficiency of the F$_1$F$_\text{ε}$-ATP synthase to function as mKATP (Figure 1). Furthermore, the cardioprotective effect of diazoxide, the canonical mKATP activator, is shown to be mediated by IF1. By applying Bayesian phylogenetic analysis, the authors conclude that IF1 is likely an ancient Bcl family member that evolved from bacteria resident in eukaryotes and prevents excessive ATP consumption through the reversal of the ATP synthase to maintain the protonmotivforce.

The authors need to be applauded for providing groundbreaking results with fundamental implications for cellular bioenergetics and survival. First, these observations identify K$^+$ import via the F$_1$F$_\text{ε}$-ATP synthase as one central mechanism by which the rate of ATP turnover in the cytosol is matched by ADP phosphorylation in mitochondria. Second, they assign the F$_1$F$_\text{ε}$-ATP synthase a central role in cardioprotection, where mitochondrial K$^+$ influx via the F$_1$/K$^+$ uniporter elevates the threshold to elicit ROS-induced permeability transition. Of note, by coupling mitochondrial K$^+$ influx to ATP production, the subsequent K$^+$ extrusion via the KHE at the expense of protonmotivforce is energetically counterbalanced, which is not the case when K$^+$ enters mitochondria via CCDC51/ABCB8 (Figure 1), thereby avoiding the production of futile heat through “uncoupled” K$^+$ leak. This led the authors to suggest that CCDC51/ABCB8-related K$^+$ flux may play a rather “fine-tuning” role compared with ATP synthase-dependent mK$_\text{ATP}$. However, since CCDC51 knock-out prevented most (but not all) of the cardioprotection provided by diazoxide, the herein suggested role of the ATP synthase as mK$_\text{ATP}$ still needs to stand the in vivo test (of time), for instance in mice deficient of IF1.

In ancient Roman myth and religion, Janus is the god of beginnings, transitions, duality and endings, deciding over war and peace, or translated to biology—over life and death. From a genetic and structural perspective, mK$_\text{ATP}$ can be defined as a Janus-faced enzyme, since in addition to its K$^+$-selective pore-forming property, mK$_\text{ATP}$ channels, located on the sarclemma and the IMM, are controlled by the metabolic state of a cell: when the cellular ATP/ADP ratio drops, activation of sarcolemmal K$_\text{ATP}$ channels hyperpolarizes the cell membrane, reducing its excitability to reduce ATP demand, whereas activation of mitochondrial K$_\text{ATP}$ channels (mK$_\text{ATP}$) optimizes ATP production through mitochondrial volume regulation, as described above. Although after its first description in the early 1990s, the electrophysiological and pharmacological properties of the mK$_\text{ATP}$ were extensively characterized, its molecular identity has long remained elusive. It was initially proposed that, akin to its sarclemma counterpart, mK$_\text{ATP}$ comprised K$^+$-selective pore-forming subunits from the Kir6.x family; however, this model was discarded as genetic ablation of Kir6.x channels did not suppress mK$_\text{ATP}$ responses. Subsequently, the renal outer medullary K$^+$ channel (ROMK) evolved as a potential pore-forming subunit of mK$_\text{ATP}$ based on a proteomic screen and in vitro evidence, but cardiac-specific knock-out of ROMK later revealed that it is dispensable for cardioprotection and mK$_\text{ATP}$ responses. Recently, a protein with previously unknown function (CCDC51) was identified as a mitochondrial pore-forming subunit of mK$_\text{ATP}$, and specific knock-out of ROMK later revealed that it is dispensable for cardioprotection and mK$_\text{ATP}$ responses.}

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Conflicts of interest
None declared in the context of this publication.

References

1. Krebs HA, Johnson WA. Metabolism of ketonic acids in animal tissues. Biochem J 1937;31(4):645–660.
2. Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 1961;191(4784):144–148.
3. Juhaszova M, Kobrinsky E, Zorov DB, et al. ATP Synthase K+ and H+-Fluxes Drive ATP Synthesis and Enable Mitochondrial K+-“Uniporter” Function: I. Characterization of Ion Fluxes. Function. 2021;3(2), doi: 10.1093/function/zqab065.
4. Feniouk BA, Kozlova MA, Knorre DA, Cherepanov DA, Mulkidjanian AY, Junge W. The proton-driven rotor of ATP synthase: ohmic conductance (10 fS), and absence of voltage gating. Biophys J 2004;86(6):4094–4109.
5. Cortassa S, Aon MA, Juhaszova M, Kobrinsky E, Zorov DB, Sollott SJ. Computational modeling of mitochondrial K+ and H+-driven ATP synthesis. J Mol Cell Cardiol 2022;(165): 9–18.
6. Juhaszova M, Kobrinsky E, Zorov DB, et al. ATP Synthase K+ and H+-Fluxes Drive ATP Synthesis and Enable Mitochondrial K+ “Uniporter” Function: II. Ion and ATP Synthase Flux Regulation. Function 2022;3(2):zqac001.
7. Juhaszova M, Zorov DB, Kim SH, et al. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 2004;113(11):1535–1549.
8. Heusch G. Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. Nat Rev Cardiol 2020;17(12):773–789.
9. Bernardi P, Carraro M, Lippe G. The mitochondrial permeability transition: recent progress and open questions. FEBS J 2021; doi: 10.1111/febs.16254.
10. Flagg TP, Enkvetchakul D, Koster JC, Nichols CG. Muscle KATP channels: recent insights to energy sensing and myoprotection. Physiol Rev 2010;90(3):799–829.
11. O’Rourke B. Evidence for mitochondrial K+ channels and their role in cardioprotection. Circ Res 2004;94(4):420–432.
12. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K+ channel in the mitochondrial inner membrane. Nature 1991;352(6332):244–247.
13. Suzuki M, Sasaki N, Miki T, et al. Role of sarcolemmal K(ATP) channels in cardioprotection against ischemia/reperfusion injury in mice. J Clin Invest 2002;109(4):509–516.
14. Foster DB, Ho AS, Rucker J, et al. Mitochondrial ROMK channel is a molecular component of mitoKATP. Circ Res 2012;111(4):446–454.
15. Papanicolaou KN, Ashok D, Liu T, et al. Global knockout of ROMK potassium channel worsens cardiac ischemia-reperfusion injury but cardiomyocyte-specific knockout does not: implications for the identity of mitoKATP. J Mol Cell Cardiol 2020;139:176–189, doi: 10.1016/j.yjmcc.2020.01.010.
16. Paggio A, Checchetto V, Campo A, et al. Identification of an ATP-sensitive potassium channel in mitochondria. Nature 2019;572(7771):609–613.
17. Ardehali H, O’Rourke B, Marbán E. Cardioprotective role of the mitochondrial ATP-binding cassette protein 1. Circ Res 2005;97(8):740–742.
18. Nath S. Two-ion theory of energy coupling in ATP synthesis rectifies a fundamental flaw in the governing equations of the chemiosmotic theory. Biophys Chem 2017;230:45–52.