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Commentary

Fantastic beasts and how to find them—Molecular identification of the mitochondrial ATP-sensitive potassium channel

Sean M. Davidson[a], Gyorgy Szabadkai[b,c], Michael R. Duchen[b,*]

[a] The Hatter Cardiovascular Institute, University College London, 67 Chenies Mews, WC1E 6HX, United Kingdom
[b] Department of Cell and Developmental Biology and UCL Consortium for Mitochondrial Research, University College London, Gower Street, London, WC1E 6BT, United Kingdom
[c] Department of Biomedical Sciences, University of Padua, Padua, Italy

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ABSTRACT

Despite reported sightings over many years, certain mitochondrial-specific channels have proven to be elusive beasts, evading molecular identification. However, combining modern genetics with a wave of their ion-sensing wand, researchers have managed to capture first the mitochondrial calcium uniporter, and now that semi-mythological beast, the mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) channel.

The recent, unequivocal identification of the gene encoding the mammalian mitochondrial K$_{ATP}$ channel (mitoK$_{ATP}$) [1] marks the culmination of nearly 30 years of dedicated investigation by many laboratories. The path has been tortuous, and the very existence of the channel has been the subject of vigorous debate [2], so the final revelation of its molecular identity is an occasion to celebrate.

Evidence for the existence of a K$_{ATP}$ channel in the inner mitochondrial membrane was first obtained by Inoue et al in 1991 [3]. In patch-clamped rat liver mitoplasts they detected a 10 pS, single-channel K^+ conductance, for which the open probability was reduced by ATP or glibenclamide, a sulphonylurea antidiabetic agent. These properties were reminiscent of those of the sarcolemmal K$_{ATP}$ channel, which, opens and hyperpolarizes the plasma membrane in response to ATP depletion. The sarcolemmal K$_{ATP}$ channel is an energy sensing pathway, coupling changes in bioenergetic status with changes in excitability. In pancreatic beta-cells, sarcolemmal K$_{ATP}$ channels play a central role in glucose-stimulated insulin secretion. In cardiomyocytes, the channels play a role in cytoprotection, sensing ATP depletion during energy depletion, driving sarcolemmal hyperpolarization and action potential shortening, protecting the cell from Ca^{2+} overload and from ATP depletion [4].

Interestingly, K^+ flux across the mitochondrial membrane seems to be more important for regulation of volume than of the mitochondrial membrane potential. For instance, when mitoK$_{ATP}$ channels open, the mitochondrial, transmembrane electrochemical gradient favours K^+ entry into the mitochondria, causing swelling and increased generation of reactive oxygen species (ROS) [5]. This action has been proposed as a mechanism underlying ischaemic preconditioning (IPC), a phenomenon whereby short periods of ischaemia and reperfusion protect the heart against subsequent prolonged ischaemia [5]. In particular, IPC leads, via a cascade of kinase pathways culminating in protein kinase G (PKG), to mitoK$_{ATP}$ opening and the production of ROS which trigger a defensive cardioprotective state, probably by inhibiting opening of the mitochondrial permeability transition pore (MPTP) [5]. Importantly, a diverse range of agents that activate these kinase pathways, including bradykinin and nitric oxide, or direct pharmacological openers of the mitoK$_{ATP}$ channel such as diazoxide, all trigger the protective state [5].

Despite the experimental evidence supporting the existence of mitoK$_{ATP}$ channels – footsteps in the sand - and some reported “near sightings” [6,7], – there has been a nagging doubt as to whether these fantastic beasts really do exist. Most reagents used to study them are not specific to the mitochondrial channel, and the bulk of evidence for a functional role of the mitoK$_{ATP}$ channel was pharmacological, based on the actions of a few non-specific compounds. Worryingly, the molecular identity of the channel could not be conclusively established. Similar doubts had previously clouded the study of the calcium uniporter (MCU) - another mitochondrial channel of semi-mythological status – until the Rizzuto and Mootha groups finally succeeded in identifying the gene encoding it in 2011 [8,9]. Once the gene was cloned, genetic techniques eventually confirmed the long-held hypothesis that mitochondrial calcium uptake contributes to IR injury [10]. Perhaps buoyed by this success, the group of Rizzuto and De Stefani have now identified the genes encoding the mitoK$_{ATP}$ [1]. Driven by scientific curiosity, the group investigated another ubiquitous and highly

* Corresponding author.
E-mail address: m.duchen@ucl.ac.uk (M.R. Duchen).

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conserved mitochondrial protein – CCDC51 (now MITOK) – which they noticed had characteristics that predict a channel structure. Overexpression of the protein in cells caused a fall in mitochondrial membrane potential ($\Delta\Psi_m$) and fragmentation. Protein reconstitution into planar lipid bilayers rewarded them with recordings demonstrating the presence of a voltage-independent K^+ channel. While the channel was ATP insensitive, ATP sensitivity of the plasma membrane K_{ATP} channels is conferred by associated SUR subunit proteins. Guided by homology, the group alighted upon ABCB8 (now referred to as MITOSUR) and showed that MITOSUR and MITOK together formed an ATP-sensitive K^+-permeable channel, which is activated by diazoxide and blocked by both glibenclamide and 5-hydroxydecanoate (5-HD).

In summary, it appears that these wizards of the mitochondrial realm have proven that the mitoK_{ATP} channel *does* indeed exist. Knockout of the channel alone caused instability of potential and decreased respiratory capacity. Knockout of the channel and regulatory subunit reduced changes in morphology in control cells in response to energy deprivation, suggesting that the channel has a 'physiological' role of driving changes in morphology in response to impaired metabolism. Importantly, the group generated mice lacking the channel and investigated its role in cardioprotection. As expected, the isolated hearts were found to be resistant to the cardioprotection by diazoxide [1]. Unfortunately, the critical question of whether IPC remains effective in these mice was not investigated here, but will no doubt answered in the next, highly anticipated sequel.

It is extremely gratifying to see research over many years reach a tangible and exciting advance. Evidence to date suggests that, while the mitoK_{ATP} channel plays a key role in many cardioprotective strategies, it must be stimulated prior to ischaemia and is not relevant once ischaemia is established – which is unfortunately the scenario for most patients with acute myocardial ischaemia. The implications will need time to be established but will be greatly facilitated by the discovery of the molecular identity of the channel and the existence of the knockout mice.

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