Commentary

MOLECULAR METABOLISM

From explosives to physiological combustion: Next generation chemical uncouplers*

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Peter Mitchell's chemiosmotic hypothesis has provided the basis for our understanding of how biological energy is transferred [1]. Chemical energy in our food is not directly used to phosphorylate ADP but it is first stored at the mitochondrial inner membrane as an electrochemical proton gradient that can be coupled to ATP synthesis (Figure 1A). The term "mitochondrial uncoupling" refers to the short-circuit in the electrochemical gradient of protons thereby releasing energy in the form of heat instead of ATP [2]. Protons may leak back to the mitochondrial matrix by either unregulated endogenous pathways, termed basal proton leak, or by regulated endogenous proteins such as uncoupling proteins (UCPs), (Figure 1A). Uncoupling can be measured directly or indirectly as a decrease in the electrochemical gradient (proton motive force) or the respiratory rate that drives proton leak, respectively. The physiological regulation of proton leak also allows for fine-tuning energy metabolism in response to environmental challenges. For example, a tightly regulated proton channel named uncoupling protein 1 (UCP1) found in brown adipose tissue has evolved in mammals to generate heat and defend body temperature during cold acclimation [3], (Figure 1A). Moreover, physiological uncoupling in other tissues enables fine-tuning of insulin secretion and protection from oxidative damage although the responsible factor(s) are not completely understood [4,5]. Therefore, targeting mitochondrial uncoupling may provide a powerful therapeutic treatment for diseases such as obesity and diabetes.

There has been a tremendous interest in understanding the physiological and pathological roles of mitochondrial function, in particular mitochondrial uncoupling, as witnessed by the over-proportional number of research studies on "mitochondria", "mitochondrial uncoupling" and "uncoupling proteins" that have been published over the last 15 years (Figure 1B). Since the beginning of the 20th century, several attempts have been made to develop pharmacological agents able to decrease metabolic efficiency by increasing mitochondrial uncoupling, commonly termed "uncouplers". The most notable, dinitrophenol (DNP) was initially used to ignite explosives during the 1st World War and later on considered as a promising drug in the war against obesity and diabetes. However, due to the narrow dose window between effective and fatal doses, serious adverse effects and lack of selectivity, DNP was soon withdrawn from the market [6,7]. Although the translational value of DNP and other chemical uncouplers such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP) and carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) remains limited, their use in biological research represents a common and fundamental analytical strategy for estimating maximal substrate oxidation in whole cells and isolated mitochondria. Surprisingly, the scientific interest in "dinitrophenol" and "FCCP" has decreased or stagnated (Figure 1C).

Chemical uncouplers are mostly lipophilic weak acids that shuttle protons from the cytosol to the matrix across the mitochondrial inner membrane but their activity cannot be acutely regulated (Figure 1A). In fact, "first-generation" uncouplers are not selective for mitochondrial membranes and show protonophoric activity on plasma membrane as well. This is especially relevant for excitatory cells where uncontrolled plasma membrane depolarization can compromise pivotal physiological functions outweighing the benefits of mitochondrial depolarization. The same applies for experiments in cell culture, which requires a careful titration of each uncoupler to find the effective dose.

In this issue of Molecular Metabolism, Kenwood et al. report on the "Identification of a novel mitochondrial uncoupler that does not depolarize the plasma membrane" [8], therefore addressing one of the most prominent downsides of protonophoric uncouplers. Through elegant bioenergetic screening methods, the authors identify a molecule named BAM15 that has uncoupling activity but lacks of cytotoxicity in intact cells. Using the patch-clamp technique they demonstrate that BAM15 uncoupling activity is selective for mitochondrial membranes, leaving the plasma membrane potential unaffected. Furthermore, initial *in vivo* experiments show that BAM15 confers protection from ischemia-reperfusion injury in kidneys. This study has finally opened a window on the use of next-generation chemical uncouplers for translational medicine and it has the potential to boost the search for analogs with therapeutic value and with limited off-target effects.

While these finding may re-ignite the research on chemical mitochondrial uncouplers with therapeutic potential, further studies will be necessary to dissect the mode of action of BAM15 and to evaluate beneficial versus adverse effects. How does a lipophilic weak acid such as BAM15 prevent off-target effects at other cellular membranes? What are the chemical properties of BAM15 mediating a selective protonophoric activity at the mitochondrial membrane? How

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DOI of original article: http://dx.doi.org/10.1016/j.molmet.2013.11.005

This commentary refers to "Identification of a novel mitochondrial uncoupler that does not depolarize the plasma membrane by kenwood et al. 10.1016/j.molmet.2013.11.005.

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Received December 31, 2013 • Accepted January 6, 2014 • Available online Month XX, 2014

http://dx.doi.org/10.1016/j.molmet.2014.01.003

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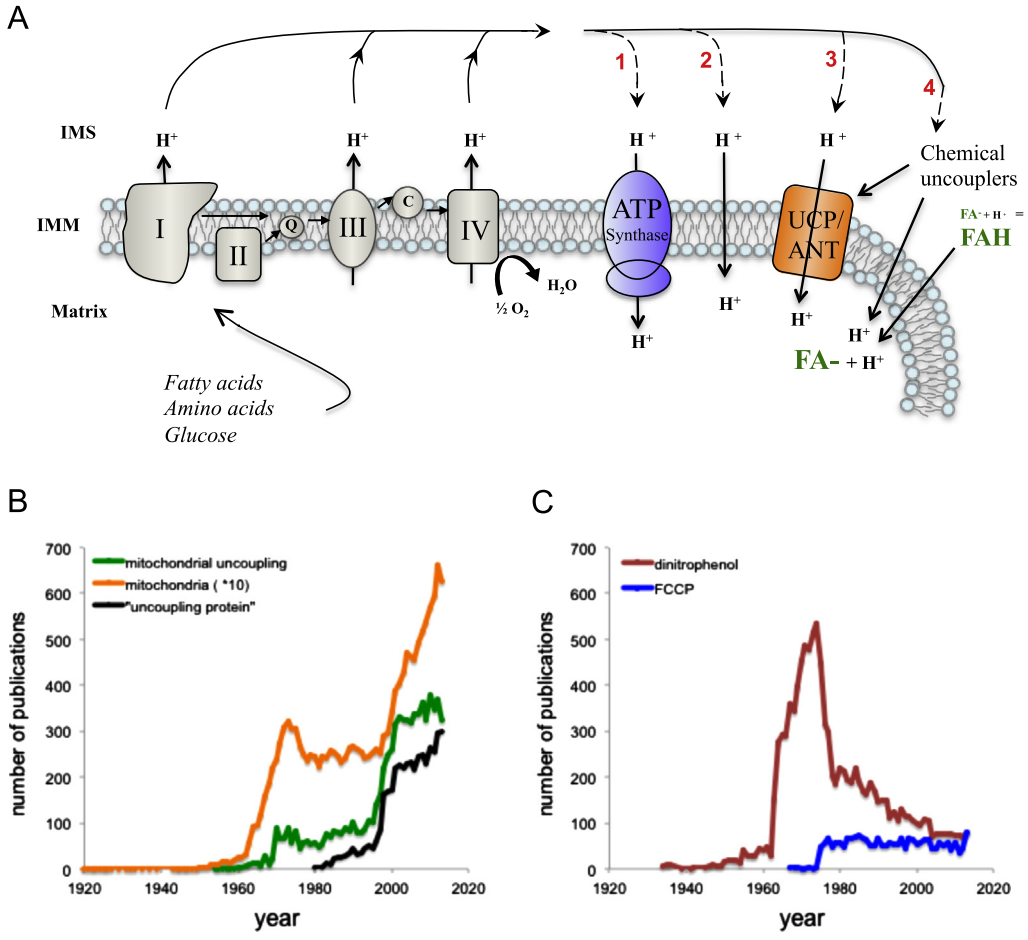

Figure 1: Mitochondrial uncoupling. (A) Schematic illustration of the mitochondrial inner membrane (IMM) and processes producing and consuming proton motive force. Respiratory chain complexes use the energy of substrate oxidation to pump protons from the matrix to the intermembrane space (IMS) and build up the proton motive force that is normally utilized to drive ATP synthesis [1]. Protons may also leak back to the mitochondrial matrix by either an unregulated basal proton leak [2] or by regulated endogenous proteins such as Uncoupling Proteins (UCPs) and the adenine nucleotide translocase (ANT), [3]. Other uncoupling agents act as weak lipophilic acids [4] or modify uncoupling action of "uncoupling" proteins [5]. By uncoupling, the proton motive force is dissipated as heat instead of being stored in ATP. (B) The graph illustrates the number of publications found in Pubmed with the keywords "mitochondria", "mitochondrial uncoupling", and "uncoupling proteins", over a period of about 100 years. (*http://www.ncbi.nlm.nih.gov/pubmed/?term=XX; December 2013*). Of note is the increasing interest in mitochondrial uncoupling during the past 10–15 years. (C) In contrast to the interest in "mitochondrial uncoupling", scientific work using "dinitrophenol" and "FCCP" decreased or stagnated, respectively.

does the potency of BAM15 relate to the potency of DNP? Caution is always required when targeting mitochondrial uncoupling via lipophilic weak acids as often their activity is not desensitized at lower proton motive force. Even when selective, lack of auto-regulation may prevent full effectiveness. Regulation of mitochondrial uncoupling through the activation of endogenous proteins with uncoupling action, such as UCPs and the adenine nucleotide translocase (Figure 1A), may provide a dose-independent, self-limiting way to uncouple mitochondria [9]. Whether BAM15 activity is self-limiting remains an open question.

The study from Kenwood and colleagues shows that the translational route of chemical mitochondrial uncoupling may not have ended 80 years ago. New methodological strategies, as the ones applied in the present study to monitor cellular bioenergetics and physiology, may prove instrumental to identify the next generation of mitochondrial uncouplers with increased translational value on common metabolic diseases.

CONFLICT OF INTEREST

None declared.

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