FUNCTION™ american physiological society

FUNCTION, 2022, 3(2): zqac004

https://doi.org/10.1093/function/zqac004
Advance Access Publication Date: 14 February 2022
Opinion Article

OPINION ARTICLE

Electrophysiological Experiments Revalidate the Two-ion Theory of Energy Coupling and ATP Synthesis

Sunil Nath *

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi 110016, India

*Address correspondence to S.N. (e-mail: sunil_nath_iit@yahoo.com; sunath@iitd.ac.in)

In a recent article in *Function* entitled "When a discovery is a rediscovery: Do we know the history of our own subject?," the Editor-in-Chief, Ole H. Petersen has brought to light the important problem of omissions and distortions in the historical record in various fields of scientific enquiry.[1] He has given the example, based on his own research experience, of Ca^{2+}-activated Cl^- channels, where misleading information on its discovery has been propagated and perpetuated. This led to the paradoxical situation where the pioneering research work of the late Sir Michael Berridge and colleagues, who first discovered the channel in 1975, almost a decade before current generally accepted narratives date the discovery, has all but disappeared from the scientific citation record.[1] In particular, Petersen has expressed his interest to hear from researchers about other cases where errors, or even deliberate omissions of key references, has distorted the general perception of key discoveries, so that a correct picture can be presented of what has been done, when, and by whom.[1]

In response to the above call, I would like to draw attention to discoveries in my own field of research on the synthesis of adenosine triphosphate (ATP), the universal energy molecule of life. This molecule was first discovered in 1929 independently by Lohmann in Germany, and by the Indian scientist SubbaRow working in Fiske's laboratory at Harvard.[2] The discovery of ATP was made almost simultaneously, as also pointed out recently by Verkhratsky et al.;[3] nonetheless, the latter authors are not given credit for the discovery. The above cases[1–3] may be dismissed in passing that these are concerned with events lost in history. However, the problems of discovery and priority continue to the present day in the field. Let me explain.

My own research work during the past three decades pertains to the molecular mechanism and thermodynamics of ATP synthesis by the F_OF_1-ATP synthase in the biological process of oxidative phosphorylation (OXPHOS) and photophosphorylation. This vital process generates >90%of the energy in the living cell. The mechanism of ATP synthesis and its hydrolysis by the myriad molecular machines of the cell has been a tantalizing puzzle ever since the discovery of ATP, one that has caused major headaches to a galaxy of scientists in the 20th century.[4] Almost all current biochemistry textbooks and articles on ATP synthesis focus exclusively on Mitchell's *one-ion* chemiosmotic theory[5] of the 1960s. Varied opinions were expressed by leading investigators in the field.[6–9] An attempt at reconciliation was made by luminaries in bioenergetics in a unique 1977 joint review that contained six separate reviews – each with a different title, emphasizing the need for clarification and further research.[10]

In this context, the process of OXPHOS was revisited in the early 1990s[2] and researched for three decades. This led to the formulation, logical development, and refinement of Nath's *two-ion* theory of energy coupling and Nath's torsional mechanism of energy transduction and ATP synthesis[2,4,11–14] – the full naming is by other book authors and researchers.[15] The theory was developed based on (i) considerations of energy balance,[2,11] (ii) the need to satisfy overall electroneutrality in reconstituted single-molecule experiments on ATP synthesis,[4] (iii) analysis by *nonequilibrium* thermodynamics[16] and kinetics,[17,18] (iv) quantitative energy landscape approaches applied to membrane transporters,[14] (v) mathematical equations for electrochemical potential by translocation of *two* ions that have been shown to accurately quantify biochemical data on ATP synthesis by reconstituted F_OF_1-ATP synthase *without using adjustable parameters*,[4] (vi) respiratory control and regulation of ATP synthesis by an integrated demand-cum-supply approach,[16] and (vii) the search for unifying principles of energy transduction in biological systems.[11,13] A key proposal of the theory is the involvement of *two* ions in ATP synthesis – and not just pro-

Submitted: 16 January 2022; **Revised:** 5 February 2022; **Accepted:** 8 February 2022

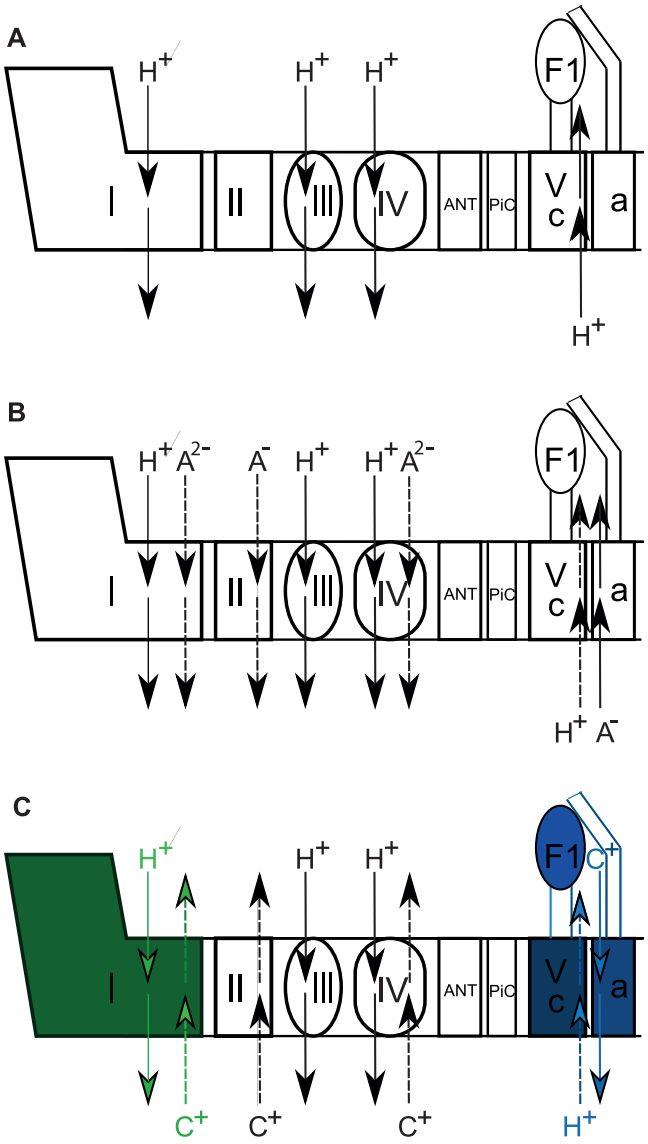

Figure 1. Concept of energy coupling in OXPHOS at the physiological level. The diagram illustrates the coupling in Complexes I – V according to Mitchell's one-ion chemiosmotic theory (A),[5] and Nath's two-ion theory, where the second ion is a dicarboxylic acid anion[2,12–14,16–18] (B), or a counter-cation such as K^+ or Na^+,[2,4,13] shown as C^+ in panel C. Primary ion translocations are denoted by bold arrows, and secondary ion translocations by dashed arrows. The part of the two-ion theory related to F_OF_1-ATP synthase (Complex V) revalidated by the recent electrophysiological experiments of Juhaszova et al.[22] is highlighted in blue, while aspects of the two-ion theory related to Complex I validated experimentally by Zemel et al.[25] are shown in green.

tons as in single-ion theories, including Mitchell's chemiosmotic theory[5] (Figure 1). The possibilities of *both* membrane-permeable anions such as succinate,[2,12,13] as well as cations such as K^+ [2,4,13] have been offered as chief candidates for the second ion, *in addition to* H^+. A detailed mechanism to realize the addition and collaborative utilization of the ions' electrochemical potential energies for ATP synthesis[2,4,12] and its regulation[16] has been formulated. Hence the two-ion theory had already extended and updated the central tenet of Mitchell's single-ion chemiosmotic theory[5] that the ATP synthase utilizes solely proton flux through F_O to synthesize ATP.

We have experimentally validated the two-ion theory through several lines of biochemical evidence. These include (i) the observed concentration dependence of succinate on the rate of ATP synthesis,[12] (ii) enzymological data on mixed inhibition by specific anion channel blockers such as the stilbene compounds,[11] (iii) tracing the path of dicarboxylic acid anion and its back-and-forth translocation across the energy-transducing membrane during photophosphorylation,[19] (iv) the kinetically pure competitive inhibition obtained with the classical weak anionic uncouplers of OXPHOS, such as 2,4-dinitrophenol, and anionic substrates/permeant ions like succinate for uptake and conversion in mitochondria,[13] and (v) studies of coupling and uncoupling of ion transport and ATP synthesis.[17,18] Succinate was identified as the *second* ion involved in physiological ATP synthesis in mitochondria.[13] The necessity of two ions for *coupling* in ATP synthesis suggests a specific molecular interpretation of OXPHOS.[14,16] Pioneering structural studies of membrane-bound transporters[20] reinforce the concept. The theory has recently been applied to

make a comprehensive energy audit of ATP consumption in the brain.[21]

Although the two-ion theory/mechanism has been published in >40 journal papers, it is often missed by lack of thorough referencing, and is therefore often not there in the citation record. There are multiple reasons for the omissions of key references in various articles. First, there is an ignorance of the previous literature in the field. Second, although the papers are known and are relevant, yet they fail to make it to the reference list either due to indifference or neglect. Third, and most egregious of all, is use of the knowledge in follow-up work, but an ethical failure to cite.

In this issue of *Function*, by use of well-designed electrophysiological single-molecule and organelle approaches, Juhaszova et al.[22,23] show that *both* H^+- and K^+-translocation through the access channels of the F_OF_1-ATP synthase drive ATP synthesis. The work experimentally[22] revalidates and computationally[24] reinforces a longstanding central tenet of the two-ion theory of energy coupling – see pp. 76, 79–80 of ref. 2, Section 5.5 in ref. 11, and refs. 4, 12–18. In another preprint of January 2022, a theoretical framework is proposed to describe how "monovalent cations contribute to the build-up of H^+ gradients and the proton motive force, extending the classical Mitchellian view on chemiosmosis."[25] Such an extension had already been formulated (Figure 1) and published previously.[2,4,13] In yet another very recent preprint,[26] the concept of frustration and symmetry mismatch in the F_1 portion of ATP synthase is rediscovered (see pp. 1792–1793 of ref. 11 and ref. 14). None of the above-mentioned works cite the original publications.

How can the problem be rectified and the record set straight? Obviously, a better system of checks and balances is needed, so that an accurate description of discovery is presented to readers.[1] One possibility is for journals to institute a separate section, as in *Function*, seeking opinions on various issues, such as of rediscovery and priority. Another is the new initiative by the web-based platform, *Qeios* that would serve to put indirect pressure on authors. A third possibility is to solicit a corrigendum. An optimistic future perspective could well visualize intelligent search engines empowering authors, reviewers, and editors to ensure completeness and authenticity of the publication record in all scientific endeavors. Thus, all major research developments, especially in interdisciplinary fields, are directed to reach the readers for a critical evaluation, thereby enabling rapid progress in science.

Conflict of Interest Statement

The author declares no conflicts of interest.

References

1. Petersen OH. When a discovery is a rediscovery: do we know the history of our own subject? *Function* 2021;**2**(4): zqab030.
2. Nath S. The molecular mechanism of ATP synthesis by F_1F_0-ATP synthase: a scrutiny of the major possibilities. *Adv Biochem Eng Biotechnol* 2002;**74**:65–98.
3. Verkhratsky A, Zimmermann H, Abbracchio MP, et al. In memoriam Geoffrey Burnstock: creator of purinergic signaling. *Function* 2020;**1**:(1):zqaa006.
4. 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.
5. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol Rev* 1966;**41**(3):445–502.
6. Massari S, Pozzan T. The accumulation ratio of K^+, Na^+, Ca^{2+} and tetrapropylammonium in steady-state mitochondria. *Arch Biochem Biophys* 1976;**173**(1): 332–340.
7. Williams RJP. Some unrealistic assumptions in the theory of chemi-osmosis and their consequences. *FEBS Lett* 1979;**102**(1):126–132.
8. Green DE. A critique of the chemosmotic model of energy coupling. *Proc Natl Acad Sci* 1981;**78**(4): 2240–2243.
9. Slater EC. The mechanism of the conservation of energy of biological oxidations. *Eur J Biochem* 1987;**166**(3): 489–504.
10. Boyer PD, Chance B, Ernster L, et al. Oxidative phosphorylation and photophosphorylation. *Annu Rev Biochem* 1977;**46**(1):955–1026.
11. Nath S. The new unified theory of ATP synthesis/hydrolysis and muscle contraction, its manifold fundamental consequences and mechanistic implications and its applications in health and disease. *Int J Mol Sci* 2008;**9**(9): 1784–1840.
12. Nath S. The torsional mechanism of energy transduction and ATP synthesis as a breakthrough in our understanding of the mechanistic, kinetic and thermodynamic details. *Thermochim Acta* 2004;**422**(1-2):5–17.
13. Nath S, Villadsen J. Oxidative phosphorylation revisited. *Biotechnol Bioeng* 2015;**112**(3):429–437.
14. Nath S. Energy landscapes and dynamics of ion translocation through membrane transporters: a meeting ground for physics, chemistry, and biology. *J Biol Phys* 2021;**47**(4):401–433.
15. Wray V. Commentary on Nath and Villadsen review entitled "Oxidative phosphorylation revisited" *Biotechnol Bioeng* 112 (2015) 429−437. *Biotechnol Bioeng* 2015;**112**(10): 1984–1985.
16. Nath S. Entropy production and its application to the coupled nonequilibrium processes of ATP synthesis. *Entropy* 2019;**21**(8):746, 1−22.
17. Nath S. Molecular mechanistic insights into coupling of ion transport to ATP synthesis. *Biophys Chem* 2018;**241**:20–26.
18. Nath S. Molecular mechanistic insights into uncoupling of ion transport from ATP synthesis. *Biophys Chem* 2018;**242**:15–21.
19. Nath S, Elangovan R. New perspectives on photosynthetic phosphorylation in the light of a torsional mechanism of energy transduction and ATP synthesis. *J Bioenerg Biomembr* 2011;**43**(6):601–610.
20. Sauer DB, Wang B, Sudar JC, et al. The ups and downs of elevator-type di-/tricarboxylate membrane transporters. *FEBS J* 2021. doi: 10.1111/febs.16158.
21. Levy WB, Calvert VG. Communication consumes 35 times more energy than computation in the human cortex, but both costs are needed to predict synapse number. *Proc Natl Acad Sci USA* 2021;**118**(18):e2008173118.
22. 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* 2022;**3**:zqab001.

23. 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**:zqab002.
24. Cortassa S, Aon MA, Juhaszova M, et al. Computational modelling of mitochondrial K^+- and H^+-driven ATP synthesis. *J Mol Cell Cardiol* 2022;**165**:9–18.
25. Zemel M, Angelin A, Potluri P, et al. The principal mitochondrial K^+ uniport is associated with respiratory complex I. *bioRxiv* 2022. Doi: https://doi.org/10.1101/2022.01.06.475251.
26. Kubo S, Niina T, Takada S. F_O-F_1 coupling and symmetry mismatch in ATP synthase resolved in every F_O rotation step. *bioRxiv* 2021. Doi: https://doi.org/10.1101/2021.11.14.468453.