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Fifty Years of Chemiosmotic Theory – Many Lights and Some Shade

Donato Pastore*

Department of Agricultural, Food and Environmental Sciences, University of Foggia, Foggia, Italy

In the grasp of life sciences the significance of bioenergetics is central and, in this framework, the chemiosmotic theory of Peter Mitchell represents the core of bioenergetics, since it highlights how the bulk of ATP synthesis occurs in living cells. According to the Mitchell's chemiosmotic theory, in mitochondria, chloroplasts and in many bacteria, the energy-rich intermediate driving ATP synthesis is the proton gradient across an energy-transducing membrane. The driving force was defined by Mitchell [1,2] as the protonmotive force, Δp, consisting of an electrical, ΔΨ, and a chemical component, ΔpH [3]. This sparkling intuition of Peter Mitchell passed over a lot of experimental confirmations and brought him the great honour of the Nobel Prize in chemistry in 1978. The chemiosmotic theory is by now 50 years old (in 2011) and really contributed to explain the major part of the pertinent experimental observations described in the literature, but a substantial set of data still occurs that escapes chemiosmosis explanations.

Should the theory be recasted in some way?

A major prediction of the chemiosmotic model is that the phosphorylation potential and the rate of ATP synthesis by oxidative phosphorylation should depend on the magnitude of the bulk Δp.

Indeed, some energy-transducing membranes were shown to trouble this statement. In *Halobacterium halobium* [4] and thylakoid vesicles [5] light-induced ATP synthesis occurs in the absence of an apparent ΔΨ or ΔpH. In extreme alkaliphilic bacteria ATP synthesis was detected even in the presence of an inverted ΔpH, alkaline outside [6]. In bovine heart submitochondrial particles the attenuation of the rate of succinate oxidation results in a parallel decrease in the rate of ATP synthesis with little or no change in Δp [7]. These findings initiated speculation as to whether the delocalized, transmembrane Δp was the principal driving force for ATP synthesis [8]. As a consequence, the idea of localized rather than delocalized energy transfer between the electron transfer complexes and the ATP synthase gained some support [9-12]. One possibility to explain these findings is that proton transfer may occur through direct protein-protein interaction [13,14]. Another possibility is that protons generated at the surface of the bilayer membrane diffuse laterally (through the polar groups at the surface of phospholipids or the organized water at the surface) [15-17]; this suggests that localized protons could be directly coupled to the phosphorylation of ADP when the protons are channelled through the ATP synthase or alternatively exchange with ions at symports or antiports [18]. Consistently, theoretical considerations [19,20] and experimental results [21] indicate that a coupling between proton donor and acceptor sites in a bilayer can be direct without involving the bulk phase with a limiting distance between the two estimated to be considerable, nanometers or micrometers depending on buffer [18]. This point is of great interest, so that one of the first papers published in this journal deals with a proton-electrostatics hypothesis for localized proton coupling bioenergetics [22].

As for intact fully functional mitochondria, the idea of a localized energy transfer between the electron transfer complexes and the ATP synthase was proposed by Tedeschi [18], who contends that in mitochondria there is no metabolically dependent ΔΨ. At this regard see the controversy Tedeschi *vs* Nicholls [18,23,24]. According to a possible localized energy transfer in intact mitochondria, very recently it has been reported that KCl-treated Durum Wheat Mitochondria (DWM) lack a measurable ΔΨ and ΔpH, but are fully coupled and are able to regularly accomplish ATP synthesis [25]. This is of particular interest since mitochondria live in an ionic cytoplasm containing about 100 mM K^+ and contain potassium transport systems that may potentially influence components of Δp *in vivo*. Consistently, the paradoxical behaviour of DWM has been connected with the ATP sensitivity of the potassium channel present in these mitochondria, that might induce a controlled collapse of Δp [25,26]. Interestingly, at my best knowledge, this is the first description of an intact mitochondrion showing simultaneously high coupling and complete collapse of the protonmotive force.

One can argue that KCl-treated DWM synthesizes ATP via oxidative phosphorylation at too low ΔΨ (about 70-100 mV in different experiments) [25], but this *in vitro* result fits well with some measurements of ΔΨ of mitochondria *in vivo*. Zhang et al. [27], who applied a new method using the combination of conventional fluorescence microscopy and three-dimensional deconvolution by exhaustive photon reassignment, measured a mitochondrial ΔΨ of about 105 mV in fibroblasts and 81 mV in neuroblastoma cells; in perfused hearts [28] and single hepatocytes [29] about 100-140 mV were measured under different metabolic conditions. As for plant cells, mitochondrial ΔΨ estimated from the subcellular ATP/ADP ratios by means of rapid subcellular fractionation of barley leaf protoplasts was calculated to be 70-95 mV under different physiological conditions [30]. So, it is clear that mitochondria show low or very low ΔΨ in living cells and that ATP can be synthesized at suboptimal ΔΨ. But the question arises about how ATP synthase may work under low force condition. ΔΨ and ΔpH are not kinetically equivalent driving forces for ATP synthase. ΔΨ represents the essential driving force for rotation of the "rotor" $\gamma\varepsilon c_n$ of the synthase; one turn of rotation of the $\gamma\varepsilon c_n$ part yields three ATP driven by the translocation of protons through c subunits [31,32]. The extent of ΔΨ required may vary as a function of H^+/ATP stoichiometry that, in turn, depends on the number of the c subunits in F_0 rotating ring. So, in mammalian mitochondria 100-120 mV are assumed to be necessary for maximal ATP synthesis (about 70-80 mV midpoint potential) by the ATP synthase having probably 9-10 c subunits, so giving calculated H^+/ATP equal to 3-3.3 [33]. Even, only 50-60 mV are sufficient for the chloroplast enzyme having 14 c subunits, so giving calculated H^+/ATP equal to 4.7 [33]. Unfortunately, so far in DWM no information is available about the number of c subunits of ATP synthase. Moreover, calculation of thermodynamic

***Corresponding author:** Pastore Donato, Department of Agricultural, Food and Environmental Sciences, University of Foggia, Via Napoli, 25 – 71122 Foggia, Italy, Tel: +39(0)881589427/32; Fax: +39(0)881587108; E-mail: d.pastore@unifg.it

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H^+/ATP stoichiometry as ΔGp/Δp in isolated mitochondria is unlikely due to an unspecific proton leak of the inner membrane, preventing a thermodynamic equilibrium [33]. However, the above data shows that ATP synthases are able to synthesize ATP at unexpected low membrane potential.

In conclusion, these "shades" do not completely oppose the "full light" of chemiosmosis, since in all described situations a proton motive force should be invoked, but it appears necessary to recast the classical model in some cases.

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