BEC BIOENERGETICS COMMUNICATIONS
Bioenerg Commun 2024.7
https://doi.org/10.26124/bec.2024-0007
Open Access

**Tributes to pioneers in bioenergetics**
BEC Series Editors:
Angelo Azzi, Erich Gnaiger

**Cite**
Devin A, Rigoulet M (2024) Tribute to Mårten Wikström: A proton pump – a new and rich paradigm in bioenergetics. Bioenerg Commun 2024.7. https://doi.org/10.26124/bec.2024-0007

**Conflicts of interest**
AD and MR declare no conflict of interest.

**Received** 2024-07-30
**Reviewed** 2024-08-23
**Revised** 2024-09-26
**Accepted** 2024-10-11
**Published** 2024-10-23

**Academic editor**
Angelo Azzi

**Reviewers**
Christopher Axelrod
Nicoleta Moisoi

# Tribute to Mårten Wikström: A proton pump – a new and rich paradigm in bioenergetics

Anne Devin[1,2], Michel Rigoulet[1,2]

1 CNRS, Institut de Biochimie et Génétique Cellulaires, UMR 5095, F-33000 Bordeaux, France
2 Université de Bordeaux, Institut de Biochimie et Génétique Cellulaires, UMR 5095, F-33000 Bordeaux, France

* Corresponding author: michel.rigoulet@ibgc.cnrs.fr

**Keywords** – cytochrome oxidase; proton pump; energy transfer yield; oxidative phosphorylation; cell growth yield

In the course of the twentieth century, a few dates stand out in the history of bioenergetics. Mitchell's theory (Mitchell 1961) established an extremely fertile new paradigm and represented a major revolution in our understanding of the vast field of energy conversion in living organisms. In the absence of precise molecular knowledge of the different oxidative phosphorylation structures of bacteria and mitochondria, Mitchell proposed a statement of energy conversion principles that were largely open to experimental probation (Mitchell 1961). A large body of literature has reported on the various approaches developed and the results obtained, which ultimately confirmed the essence of Mitchell's hypothesis (Mitchell, Moyle 1969; Nicholls 1974a; Nicholls 1974b).

However, as far as the respiratory chain was concerned, one point remained unsatisfactory. The functional organization into redox loops imposed two predictable consequences: an $H^+$/2e- value of 2 per "site", i.e. each effective redox loop in the respiratory chain and cytochrome *c* oxidase was merely an electron transporter. The first point was challenged by the pioneering work of Brand et al, who measured a higher $H^+$/O than initially estimated (Brand et al 1976). The underestimation of the $H^+$/O quotient determined by Moyle and Mitchell (Moyle, Mitchell 1978) using the oxygen pulse technique has been confirmed by many other authors. This underestimation has been proposed to be due to the electroneutral proton phosphate transporter (Brand et al 1976; Pozzan et al 1979).

The major breakthrough came when Mårten Wikstrom demonstrated that cytochrome *c* oxidase functions as a proton pump (Wikström 1977). His very elegant and

convincing work proved that the redox loop is not a unique means of extracting protons from the matrix into the intermembrane space of mitochondria. In Mitchell's scheme, the redox loop dictates that only electrons are transferred from cytochrome *c* to oxygen. Consequently, the demonstration that cytochrome *c* oxidase is a proton pump escapes Mitchell's redox loop. It has shown that the respiratory chain cannot be organized as a redox loop. This represents a major advance in our understanding of the structure-function of the respiratory chain.

Indeed, while the redox loop determines a strict stoichiometry between proton extrusion and electron transfer (2 per site) as in a chemical reaction, the proton pump concept changed this view. In such a proton pump acting as a common physical device, electron transfer was indirectly associated with proton vector motion, and so the $H^+/2e^-$ yield could be variable depending on the value of the flux, the forces involved and possibly the kinetic effectors. The long debate between Peter Mitchell and Mårten Wikström and finally Mitchell's acceptance (Mitchell et al 1985) of the concept of cytochrome *c* oxidase as a proton pump was a major step forward in the progression of thinking and understanding of mechanisms in the bioenergetics community. It is beyond the scope of this brief review to list all the scientists involved in experiments that supported either of these two hypotheses. Wikström's work opened up two important fields of bioenergetics.

Firstly, the demonstration that not only cytochrome *c* oxidase but also complex I of the respiratory chain (Galkin et al 1999) and ATP synthase (Alexandre et al 1978) are proton pumps led to very active research into the proton channels concerned, which in turn led to intensive structural and functional studies. We can also claim that Wikström's pioneering work and research to precisely define the two proton channels in cytochrome *c* oxidase (see Wikström et al 2023 for a review) has had a major influence on the generation of biochemists who have studied structure/function relationships in other oxidative phosphorylation complexes: complex I (Hunte et al 2010) and ATP synthase (Spikes et al 2020; Boyer 1998).

Secondly, in each of the proton pumps, the coupling mechanism may be original, and many efforts have been and are being developed to understand each process involved. These two issues are, of course, closely linked. Another question raised by indirect coupling in proton pumps is the actual yield of these energy conversion processes (Luvisetto et al 1991; Pietrobon et al 1983; Pietrobon, Caplan 1985). Indeed, such engines cannot escape physical laws, and the efficiency of energy transfer must depend on the associated forces (redox, $\Delta\Psi$, $\Delta pH$) and the value of the proton and electron fluxes passing through the complexes (I and IV). Kinetic rules may also influence the efficiency of energy conversion. Although there is no doubt about these assertions, the contribution of intrinsic uncoupling (called slipping by the Azzone group, see Pietrobon et al 1983; Luvisetto et al 1991) to the overall waste of energy during oxidative phosphorylation has remained an open question. This point may be important from a physiological and pathological point of view, and unfortunately remains largely obscured in popular biochemistry literature.

The yield of oxidative phosphorylation has been extensively studied, both in isolated mitochondria and in whole cells. In isolated mitochondria, ADP/O and ATP/O ratios were found to be very similar, depending on the respiratory substrate and the number of proton pumps involved (Lemasters 1984). These studies were carried out under experimental conditions of high flux and saturating amounts of the respiratory substrates ADP and $P_i$. However, it has been shown in yeast mitochondria that if respiratory flux

were modulated by respiratory substrate concentration with the same number of proton pumps (2 in this case), the ATP/O ratio (yield) would increase when respiratory flux decreased (Fitton et al 1994). This raises the question of what type of whole-cell regulation would maintain a constant ATP/O ratio. Indeed, most of the time, it has been shown that this yield is constant in whole cells.

In living cells, growth is the result of a coupling between substrate catabolism and multiple metabolic processes that occur during net biomass formation and maintenance of cell properties. A crucial parameter for describing growth is its yield, i.e. the efficiency of the conversion from substrate consumption to biomass formation. Using a large number of yeast strains growing on different respiratory media, we have shown that growth yield is virtually the same whatever the strain, growth phase and respiratory substrate used (Devin et al 2006). This homeostasis is the consequence of a strict linear relationship between growth and respiratory rates. Furthermore, under all the conditions tested, the rate of oxygen consumption is strictly controlled by the cellular content of respiratory chain compounds, so that in vivo, the steady state of oxidative phosphorylation is maintained constant. Thus, the homeostasis of growth enthalpy depends on the close adjustment of the cellular content of respiratory chain compounds to the growth rate. Taking into account the proportion of energy used for maintenance, we can conclude that mitochondria alone constitute the main heat dissipation system in a fully aerobic metabolism, and that the decrease in the quantity of mitochondria when the growth rate decreases leads to a constant enthalpic growth yield. Thus, any process that leads to a defect in this adaptation allows energy to be wasted and consequently reduces energy yield (Devin et al 2006; Dejean et al 2002; Nogueira et al 2002; Piquet et al 2000; Leverve et al 1998; Rigoulet et al 1998).

Since adaptation to physiological changes in cellular energy demand is a critical requirement for life, mitochondrial oxidative phosphorylation is tightly controlled by ATP consumption. However, the mechanisms by which such large variations in ATP synthesis capacity can occur and the consequences for the overall efficiency of oxidative phosphorylation are not known. By studying several in vivo physiological models in rats (hyper- and hypothyroidism, polyunsaturated fatty acid deficiency and chronic ethanol intoxication - Nogueira et al 2002; Piquet et al 2000; Nogueira et al 2001), we found that the increase in hepatocyte respiration (from 9.8 to 22.7 nmol $O_2$/(min x mg dry cells)) was closely correlated with total mitochondrial cytochrome content, expressed either per mg dry cells or per mg mitochondrial protein. Moreover, this increase in total cytochrome content is accompanied by an increase in the corresponding proportion of cytochrome *c* oxidase; while total cytochrome content is multiplied by 2, cytochrome *c* oxidase is multiplied by 10. This change is associated with a reduction in the overall efficiency of the respiratory chain. As cytochrome *c* oxidase is well known for its ability to switch between redox reactions and proton pumping, we proposed that this dramatic increase in cytochrome *c* oxidase was responsible for the decrease in overall respiratory chain efficiency and hence the yield of ATP synthesis, linked to the adaptive increase in oxidative phosphorylation capacity.

Wikström's work, and in particular his demonstration that cytochrome *c* oxidase is a proton pump, had a considerable impact on our research and on the scientific questions we decided to tackle. He was a pioneer in the study of mitochondrial oxidative phosphorylation. For us, he has made the most important contribution to the field of bioenergetics since Mitchell's pioneering work.

**Acknowledgements**

This work was supported by the CNRS (Conseil National de la Recherche Scientifique), The ANR and the Fondation Guérir du Cancer, hosted by la Fondation de France.

**Abbreviations**

| | | | |
|---|---|---|---|
| ATP | adenosine triphosphate | $O_2$ | oxygen |
| ADP | adenosine diphosphate | $\Delta\Psi$ | electrical difference across the mitochondrial membrane |
| $H^+$ | proton | $\Delta pH$ | chemical difference across the mitochondrial membrane |
| e- | electron | $P_i$ | inorganic phosphate |

## References

Alexandre A, Reynafarje B, Lehninger AL (1978) Stoichiometry of vectorial $H^+$ movements coupled to electron transport and to ATP synthesis in mitochondria. https://doi.org/10.1073/pnas.75.11.5296

Boyer PD (1998) ATP synthase–past and future. https://doi.org/10.1016/s0005-2728(98)00066-8

Brand M D, Reynafarje B, Lehninger A L ( 1976) Re-evaluation of the $H^+$/site ratio of mitochondrial electron transport with the oxygen pulse technique. https://doi.org/10.1016/S0021-9258(17)33110-1

Dejean L, Beauvoit B, Alonso AP, Bunoust O, Guérin B, Rigoulet M (2002) cAMP-induced modulation of the growth yield of *Saccharomyces cerevisiae* during respiratory and respiro-fermentative metabolism. https://doi.org/10.1016/s0005-2728(02)00240-2

Devin A, Dejean L, Beauvoit B, Chevtzoff C, Avéret N, Bunoust O, Rigoulet M (2006) Growth yield homeostasis in respiring yeast is due to a strict mitochondrial content adjustment. https://doi.org/10.1074/jbc.M604800200

Fitton V, Rigoulet M, Ouhabi R, Guérin B (1994) Mechanistic stoichiometry of yeast mitochondrial oxidative phosphorylation. https://doi.org/10.1021/bi00198a039

Galkin AS, Grivennikova VG, Vinogradov AD (1999) $H^+$/2e- stoichiometry in NADH-quinone reductase reactions catalyzed by bovine heart submitochondrial particles. https://doi.org/10.1016/S0014-5793(99)00575-X

Hunte C, Zickermann V, Brandt U. (2010) Functional modules and structural basis of conformational coupling in mitochondrial complex I. https://doi.org/10.1126/science.1191046

Lemasters JJ (1984) The ATP-to-oxygen stoichiometries of oxidative phosphorylation by rat liver mitochondria. An analysis of ADP-induced oxygen jumps by linear nonequilibrium thermodynamics. https://pubmed.ncbi.nlm.nih.gov/6548475/

Leverve X, Sibille B, Devin A, Piquet MA, Espié P, Rigoulet M (1998) Oxidative phosphorylation in intact hepatocytes: quantitative characterization of the mechanisms of change in efficiency and cellular consequences. https://doi.org/10.1023/A:1006810209531

Luvisetto S, Conti E, Buso M, Azzone GF (1991) Flux ratios and pump stoichiometries at sites II and III in liver mitochondria. Effect of slips and leaks. http://www.ncbi.nlm.nih.gov/pubmed/1845985

Mitchell P (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. https://doi.org/10.1038/191144a0

Mitchell P, Moyle J (1969) Estimation of membrane potential and pH difference across the cristae membrane of rat liver mitochondria. https://doi.org/10.1111/j.1432-1033.1969.tb19633.x

Mitchell P, Mitchell R, Moody AJ, West IC, Baum H, Wrigglesworth JM (1985) Chemiosmotic coupling in cytochrome oxidase: Possible protonmotive O loop and O cycle mechanisms. https://doi.org/10.1016/0014-5793(85)80863-2

Moyle J and Mitchell P (1978) Cytochrome *c* oxidase is not a proton pump. https://doi.org/10.1016/0014-5793(78)80190-2

Nicholls DG (1974) The influence of respiration and ATP hydrolysis on the proton-electrochemical gradient across the inner membrane of rat-liver mitochondria as determined by ion distribution. https://doi.org/10.1111/j.1432-1033.1974.tb03899.x

Nicholls DG (1974) Hamster brown-adipose-tissue mitochondria. The control of respiration and the proton electrochemical potential gradient by possible physiological effectors of the proton conductance of the inner membrane. https://doi.org/10.1111/j.1432-1033.1974.tb03861.x

Nogueira V, Piquet MA, Devin A, Fiore C, Fontaine E, Brandolin G, Rigoulet M, Leverve XM (2001) Mitochondrial adaptation to in vivo polyunsaturated fatty acid deficiency: increase in phosphorylation efficiency. https://doi.org/10.1023/a:1005624707780

Nogueira V, Walter L, Avéret N, Fontaine E, Rigoulet M, Leverve XM (2002) Thyroid status is a key regulator of both flux and efficiency of oxidative phosphorylation in rat hepatocytes. https://doi.org/10.1023/a:1013822820840

Pietrobon D, Zoratti M, Azzone GF (1983) Molecular slipping in redox and ATPase $H^+$ pumps. https://doi.org/10.1016/0005-2728(83)90131-7

Pietrobon D, Caplan SR (1985) Flow-force relationships for a six-state proton pump model: intrinsic uncoupling, kinetic equivalence of input and output forces, and domain of approximate linearity. https://doi.org/10.1021/bi00342a012

Piquet MA, Nogueira V, Devin A, Sibille B, Filippi C, Fontaine E, Roulet M, Rigoulet M, Leverve XM (2000 ) Chronic ethanol ingestion increases efficiency of oxidative phosphorylation in rat liver mitochondria. https://doi.org/10.1016/s0014-5793(00)01225-4

Pozzan T, Miconi V, Di Virgilio F, Azzone GF (1979) $H^+$/site, charge/site, and ATP/site ratios at coupling sites I and II in mitochondrial e- transport. https://pubmed.ncbi.nlm.nih.gov/39939/

Rigoulet M, Devin A, Espié P, Guérin B, Fontaine E, Piquet MA, Nogueira V, Leverve X (1998) Flux-force relationships in intact cells: a helpful tool for understanding the mechanism of oxidative phosphorylation alterations? https://doi.org/10.1016/s0005-2728(98)00051-6

Spikes TE, Montgomery MG, Walker JE (2020) Structure of the dimeric ATP synthase from bovine mitochondria. https://doi.org/10.1073/pnas.2013998117

Wikström M K F (1977) Proton pump coupled to cytochrome *c* oxidase in mitochondria. https://doi.org/10.1038/266271a0

Wikström M, Gennis RB, Rich PR (2023) Structures of the intermediates in the catalytic cycle of mitochondrial cytochrome *c* oxidase. https://doi.org/10.1016/j.bbabio.2022.148933

**Copyright** © 2024 The authors. This Open Access peer-reviewed communication is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited. © remains with the authors, who have granted BEC an Open Access publication license in perpetuity.