Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

SUPPLEMENTARY INFORMATION IN DEFENSE OF MURBURN EXPLANATION FOR AEROBIC RESPIRATION

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Item 1: How six decades of chemiosmosis has sent scientists on vain consensus seeking exercises and not given oxygen its due!

Mitchell said that Comp. IV is not a proton pump!

Volume 88, number 2

FEBS LETTERS

April 1978

CYTOCHROME *c* OXIDASE IS NOT A PROTON PUMP

Jennifer MOYLE and Peter MITCHELL

Glynn Research Laboratories, Bodmin, Cornwall, PL30 4AU, England

Received 23 January 1978

1. Introduction

Mitchell postulated that cytochrome *c* oxidase is plugged through the coupling membrane of mitochondria and bacteria so that the reduction of O_2 to $2 H_2O$ involves the translocation of $4 e^-$ from cytochrome *c* at the outer surface of the membrane to $4 H^+$ ions that enter the reaction domain from the inner aqueous phase [1]. As evidence for this putative

matter whether O_2 or ferricyanide was used as oxidant [2]. As ferricyanide is impermeant and oxidises cytochrome *c*, bypassing cytochrome *c* oxidase, we concluded that cytochrome *c* oxidase does not act as a proton pump, but catalyses only the net translocation of electrons [3]. Work by Hinkle, Papa, Racker and others, using mitochondria, sonically-prepared mitochondrial vesicles and liposomes inlaid with cytochrome *c* oxidase has greatly strength-

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Nałęcz said Complex III is not a proton pump!

Journal of Bioenergetics and Biomembranes, Vol. 18, No. 1, 1986

MINI REVIEW

Is There Sufficient Experimental Evidence to Consider the Mitochondrial Cytochrome *bc₁* Complex a Proton Pump? Probably No.¹

Maciej J. Nałęcz²

Received October 8, 1985

Judy Hirst says Comp. I is a sodium pump!

www.pnas.org/cgi/doi/10.1073/pnas.0330050100

PNAS | February 4, 2003 | vol. 100 | no. 3 | 773–775

The dichotomy of complex I: A sodium ion pump or a proton pump

Judy Hirst*

Medical Research Council, Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, United Kingdom

Complex I (NADH/quinone oxidoreductase) is the only enzyme of the membrane-bound respiratory chain to remain a “black box.” Atomic resolution structural models are available for three of the four energy-transducing respiratory complexes: the cytochrome *bc₁* complex, cytochrome oxidase, and the *F₁* domain of ATP synthase. Detailed mechanisms that integrate these structural models with the results of spectroscopic and functional studies are therefore being constructed (1). In comparison, due to its large size and subunit complexity, and because its cofactors are difficult to distinguish spectroscopically, complex I has proved intractable. The complex is

prise the simpler bacterial enzymes (9) and therefore are deemed sufficient for catalysis and energy transduction. The core subunit conservation and homology suggest that all complexes I operate by a common mechanism. However, if bovine complex I pumps four protons across the membrane for each NADH oxidized but complex I from *K. pneumoniae* pumps two sodium ions (10), then this conclusion must be questioned. These different cation specificities and stoichiometries have fundamental implications for our understanding of the mechanism of complex I and its role in energy transduction and metabolism, and challenge the relevance of bacterial complexes I as models for the mamma-

allow meaningful sequence comparisons to be drawn, and these may help to identify the cation-binding sites. A similar approach was employed successfully for ATP synthase (11): most ATP synthases use a proton motive force, but closely related examples that use a sodium ion motive force have been identified in bacteria such as *Propionigenium modestum*. A family of sodium ion/proton antiporters identified in alkaliphilic bacteria contain stretches of sequence similarity with the membrane-bound ND2, 4, and 5 (NuoN, -M, and -L) subunits of complex I (12, 13). On this basis, these subunits have been proposed to bind cations and to constitute a conforma-

Leonid Sazanov says Comp. I is a proton pump!

Nature Reviews Molecular Cell Biology | AOP, published online 20 May 2015; doi:10.1038/nrm3997

REVIEWS

A giant molecular proton pump: structure and mechanism of respiratory complex I

Leonid A. Sazanov

Abstract | The mitochondrial respiratory chain, also known as the electron transport chain (ETC), is crucial to life, and energy production in the form of ATP is the main mitochondrial function. Three proton-translocating enzymes of the ETC, namely complexes I, III and IV, generate proton motive force, which in turn drives ATP synthase (complex V). The atomic structures and basic mechanisms of most respiratory complexes have previously been established, with the exception of complex I, the largest complex in the ETC. Recently, the crystal structure of the entire complex I was solved using a bacterial enzyme. The structure provided novel insights into the core architecture of the complex, the electron transfer and proton translocation pathways, as well as the mechanism that couples these two processes.

Voet's Biochemistry ratios (Comp. I = 4, CoQ = 4, Comp. IV = 2)

834 Chapter 22. Electron Transport and Oxidative Phosphorylation

Figure 22-14 The mitochondrial electron-transport chain. The pathways of electron transfer (black) and proton pumping (red) are indicated. Electrons are transferred between Complexes I and III by membrane-soluble CoQ (Q) and between Complexes

III and IV by the peripheral membrane protein cytochrome *c* (Cyt *c*). Complex II (not shown) transfers electrons from succinate to CoQ. See the Animated Figures

Lehningers' Principles of Biochemistry ratios (Comp. I = 4, Comp. III = 4, Comp. IV = 2)

(Same as Garret-Grisham's & Stryer's textbooks)

19.2 ATP Synthesis 705

FIGURE 19-17 Chemiosmotic model. In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient ($\Delta\psi$). The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (F_0). The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F_1 complex associated with F_0 .

Metzler's Biochemistry ratios (Comp. I = 4, Comp. III = 4, Comp. IV = 4)

Figure 18-5 A current concept of the electron transport chain of mitochondria. Complexes I, III, and IV pass electrons from NADH or NADPH to O₂, one NADH or two electrons reducing one O to H₂O. This electron transport is coupled to the transfer of about 12 H⁺ from the mitochondrial matrix to the intermembrane space. These protons flow back into the matrix through ATP synthase (V), four H⁺ driving the synthesis of one ATP. Succinate, fatty acyl-CoA molecules, and other substrates are oxidized via complex II and similar complexes that reduce ubiquinone Q, the reduced form QH₂ carrying electrons to complex III. In some tissues of some organisms, glycerol phosphate is dehydrogenated by a complex that is accessible from the intermembrane space.

Wikstrom, PNAS 2012 (Comp. I = 3, Comp. III = 2, Comp. IV = 4)

Table 1. Conversion of measured ATP/2 e⁻ ratios to proton translocation stoichiometries in respiratory chain of rat-liver mitochondria

Complex	ATP/2 e ⁻	H ⁺ /2 e ⁻ (efficiency,%)
I + III + IV	2.27 ± 0.08	8.32 (92%)
III + IV	1.48 ± 0.04	5.43 (91%)
III	0.49 ± 0.02	1.80 (90%)
IV	0.98 ± 0.09	3.59 (90%)
I (I + III + IV minus III + IV)	0.79	2.90 (97%)
I (I + III + IV minus III minus IV)	0.80	2.90 (97%)

The ATP/2 e⁻ ratios are from Hinkle et al. (20) for rat-liver mitochondria oxidizing β-OH-butyrate (I + III + IV) or succinate (III + IV) by O₂, succinate by ferricyanide (III), or TMPD (+ascorbate) by O₂ (IV). The H⁺/2 e⁻ ratios are derived from the ATP/2 e⁻ ratio using Eq. 4 and a value of 3.67 for the H⁺/ATP ratio. Per cent efficiency is reported for the assumption that the effective mechanistic H⁺/2 e⁻ stoichiometries⁷ are 3, 2, and 4, respectively, for complexes I, III, and IV (i.e., 9 for I + III + IV and 6 for III + IV). The two ATP/2 e⁻ ratios for complex I are derived from the measured ratios, as indicated.

Wikstrom, Chem Rev 2015 (Comp. I = 3-4, Comp. III = 4, Comp. IV = 2)

Chemical Reviews

Review

Figure 1. Respiratory chain and ATP synthase. The respiratory chain is depicted in a phospholipid membrane, as it is found in most mitochondria and in many bacteria. ATP synthase is shown to translocate 8 protons per 3 synthesized ATP molecules, as observed in animal mitochondria.

The ways in which the number of protons are transferred/cycled are also different!

Sazanov's mechanism, Nature Reviews, 2015

Antony Crofts's mechanism (his website: <https://www.life.illinois.edu/crofts/bioph354/lect8.html>)

Some other researchers' publications from the Asian subcontinent ↓

Sun F et al. (Rao Zihe group) Curr. Opin. Str. Biol., 2013 (Comp. I = 4, Comp. III = 4, Comp. IV = 2)

Zhao R-Z et al. (Zhi-Bin Yu group), Int. J. Mol. Med., 2019 (Comp. I = 2, Comp. III = 4, Comp. IV = 4)

Figure 1 - Generation of electron leaks and proton leaks in the electron transport chain. Electrons derived from oxidizable substrates are passed through CI/III/IV or CII/III/IV in an exergonic process that drives the proton pumping into the IMS of CI, CIII and CIV. The energy of the proton gradient drives the ATP synthesis of CV or can be consumed by UCPs. The sites of superoxide production in each complex are also indicated, including sites IF and IQ in CI, sites IIF in CII and site IIIQo in CIII. The O₂⁻ released into the IMS by site IIIQo can be converted into H₂O₂ in a reaction catalyzed by superoxide dismutase 1 and H₂O₂ then may diffuse into the cytoplasm.

Consensus formation efforts in recent times in the western hemisphere↓

Olivier Biner et al., Chimia, 2018 (Comp. I = 4, Comp. III = 2, Comp. IV = 4)

Fig. 1. Schematic representation of the respiratory chain in a mitochondrion. Complex I (PDB: 4WZ7) oxidizes NADH to NAD⁺ to reduce ubiquinone (Q) to ubiquinol (QH₂). The electron transfer is coupled to the transport of four protons from the mitochondrial matrix to the inner membrane space (IMS). Complex II (PDB: 2WDV) oxidizes succinate to fumarate and reduces Q without proton translocation. The electrons are then transferred from QH₂ to cytochrome c via complex III accompanied by the translocation of two protons. Finally, four molecules of cytochrome c are used to reduce oxygen to water in complex IV coupled to the transport of four protons. Overall, a total of ten protons are translocated across the mitochondrial membrane per NADH oxidized. The electrochemical proton gradient is dissipated by the ATP synthase to generate ATP. Electron and proton transfer processes are depicted with red and blue arrows, respectively.

Lewis MT – Kasper JD – Bazil JN – Frizbee JC – Wiseman RW, Int. J. Mol. Sci., 2019 (Comp. I = 4, Comp. III = 2, Comp. IV = 4)

Same status prevails in chloroplast ETC-CRAS!!

Quinone pumps protons, PS II is not a proton pump!

[https://bio.libretexts.org/Bookshelves/Biochemistry/Book%3A_Biochemistry_Free_For_All_\(Ahern%2C_Rajagopal%2C_and_Tan\)/05%3A_Energy/5.03%3A_Energy_-_Photophosphorylation](https://bio.libretexts.org/Bookshelves/Biochemistry/Book%3A_Biochemistry_Free_For_All_(Ahern%2C_Rajagopal%2C_and_Tan)/05%3A_Energy/5.03%3A_Energy_-_Photophosphorylation)

Cyt. b_6f pumps protons with quinones, PS II is a partial proton pump!

Figure 12-37
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

Both PS II and Cyt. b_6f pump protons!

Figure 19-25
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

Item 2: False attributions on lack of citations

In both the papers of KMM that PJS cites, KMM had critiqued the inferences drawn from data from duly cited reputed sources of textbooks and reviews.

The first paper's

Aerobic Respiration: Criticism of the Proton-centric Explanation Involving Rotary Adenosine Triphosphate Synthesis, Chemiosmosis Principle, Proton Pumps and Electron Transport Chain

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very first page-

the primary drive for ATP-synthesis. Since 1980s to till date, chemiosmosis or proton-centric coupling became the mainstay explanation for mOxPhos in leading reviews^{16,17} and textbooks.¹⁻³ This acclaimed explanation comprises of the fol-

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2. Berg JM, Tymoczko JL, Stryer L. *Biochemistry*. 5th ed. New York, NY: W. H. Freeman; 2002.
3. Voet D, Voet JG. *Biochemistry*. 4th ed. Hoboken, NJ: Wiley; 2011.
16. Junge W, Nelson N. ATP synthase. *Annu Rev Biochem*. 2015;84:631–657.
17. Mukherjee S, Warshel A. The F₀F₁ ATP synthase: from atomistic three-dimensional structure to the rotary-chemical function. *Photosynthesis Res*. 2017;134:1–15.

The second paper's

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
<https://doi.org/10.1080/07391102.2018.1552896>

Taylor & Francis
Taylor & Francis Group

Aerobic respiration: proof of concept for the oxygen-centric murburn perspective

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first and second pages

ive oxygen species (DROS, a perceived hazard/waste). The long-standing mechanistic explanation for cellular respiration or mitochondrial oxidative phosphorylation (mOxPhos), as originally proposed and advocated in biochemistry textbooks and recent reviews (Berg, Tymoczko, & Stryer, 2002; Boyer, 1997; Junge & Nelson, 2015; Lehninger, Nelson, & Cox, 2004; Mitchell, 1961; Mukherjee & Warshel, 2017; Voet & Voet, 2011), solicits an elaborate 'proton-centric' dynamic scheme involving 'Rotary ATP-synthesis – Chemiosmosis principle – Proton pumps – Electron Transport Chain (ETC)' (henceforth abbreviated RCPE). Several seasoned researchers have doubted many aspects of the RCPE paradigm, even after the RCPE proposal was recognized with Nobels (Berden, 2003; Ling, 1981; Nałecz, 1986; Nath, 2010; Slater, 1987; Wainio, 1985; Williams, 1979). Also, recent crystal structures of mam-

Item 3: Here is the content from Voet textbook that we quoted to critique the energetic non-feasibility.

Given that proton pumping requires 20-22 kJ/mol and heterolysis of water requires ~79 kJ/mol, as per all the texts, our quantitative analysis of the failure of CRAS is accurate!

830 Chapter 22 Electron Transport and Oxidative Phosphorylation

Complex I catalyzes oxidation of NADH by CoQ:

Complex III catalyzes oxidation of CoQ (reduced) by cytochrome c:

Complex IV catalyzes oxidation of cytochrome c (reduced) by O_2 , the terminal electron acceptor of the electron-transport process:

The changes in standard reduction potential of an electron pair as it successively traverses Complexes I, III, and IV correspond, at each stage, to sufficient free energy to power the synthesis of nearly one ATP molecule.

Complex II catalyzes the oxidation of FADH_2 by CoQ.

This redox reaction does not release sufficient free energy to synthesize ATP; it functions only to inject the electrons from FADH_2 into the electron-transport chain.

Item 4: The data on binding constants of Complex V that PJS disclaimed quoting that the relevant texts did not have this content.

Table 4. Compilation of equilibrium and rate terms for the physiological functioning of Complex V.

No.	Term	Unit	ATP synthesis		ATP hydrolysis	
			ADP	Pi	ATP	H ₂ O
1	Concentration	M	$\sim 10^{-4}$	$\sim 10^{-2}$	$\leq 10^{-3}$	$> 10^1$
2	Affinity, K_d	M	$\sim 10^{-5}$	$> 10^{-2}$	$\leq 10^{-12}$	—
3	ak_{on}	s^{-1}	$\sim 10^3$	$\sim 10^6$	$\leq 10^{-4}$	—
4	bActivity	—	$\sim 10^1$	$\leq 10^0$	$> 10^9$	$\sim 10^0$
5	cRate index	—	$< 10^1$	—	$\sim 10^9$	—

^aCalculated from K_d assuming a diffusion-limited second-order on-rate of $10^8 \text{ M}^{-1} \text{ s}^{-1}$.

^bDividing (1) with (2).

^cMultiplying the terms in (4).

The data is duly given in Lehninger Text, as highlighted below.

Kinetic studies of the initial rates of ATP synthesis and hydrolysis confirm the conclusion that $\Delta G'^{\circ}$ for ATP synthesis on the enzyme is near zero. From the measured rates of hydrolysis ($k_1 = 10 \text{ s}^{-1}$) and synthesis ($k_{-1} = 24 \text{ s}^{-1}$), the calculated equilibrium constant for the reaction

is

$$K'_{\text{eq}} = \frac{k_{-1}}{k_1} = \frac{24 \text{ s}^{-1}}{10 \text{ s}^{-1}} = 2.4$$

From this K'_{eq} , the calculated apparent $\Delta G'^{\circ}$ is close to zero. This is much different from the K'_{eq} of about 10^5 ($\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$) for the hydrolysis of ATP free in solution (not on the enzyme surface).

What accounts for the huge difference? ATP synthase stabilizes ATP relative to $\text{ADP} + \text{P}_i$ by binding ATP more tightly, releasing enough energy to counterbalance the cost of making ATP. Careful measurements of the binding constants show that F_0F_1 binds ATP with very high affinity ($K_d \leq 10^{-12} \text{ M}$) and ADP with much lower affinity ($K_d \approx 10^{-5} \text{ M}$). The difference in K_d corresponds to a difference of about 40 kJ/mol in binding energy, and this binding energy drives the equilibrium toward formation of the product ATP.