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

Kelath Murali Manoj

Satyamjayatu: The Science & Ethics Foundation, Kulappully, Shoranur, India.

ABSTRACT: The acclaimed explanation for mitochondrial oxidative phosphorylation (mOxPhos, or cellular respiration) is a deterministic proton-centric scheme involving four components: Rotary adenosine triphosphate (ATP)-synthesis, Chemiosmosis principle, Proton pumps, and Electron transport chain (abbreviated as RCPE hypothesis). Within this write-up, the RCPE scheme is critically analyzed with respect to mitochondrial architecture, proteins' distribution, structure-function correlations and their interactive dynamics, overall reaction chemistry, kinetics, thermodynamics, evolutionary logic, and so on. It is found that the RCPE proposal fails to explain key physiological aspects of mOxPhos in several specific issues and also in holistic perspectives. Therefore, it is imperative to look for new explanations for mOxPhos.

KEYWORDS: respiratory proteins, mitochondrial membrane, heme/flavin proteins, ATP-synthesis, electron transport chain

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CORRESPONDING AUTHOR: Kelath Murali Manoj, Satyamjayatu: The Science & Ethics Foundation, Kulappully, Shoranur-2 (PO) 679122, Kerala, India. Email: satyamjayatu@yahoo.com

A Survey of the Various Views on Aerobic Cellular Respiration

Erroneous perceptions prevailed at the dawn of 20th century that mitochondria, "minute observations in microscopy", were parasitic infections of cells. By mid-20th century, the efforts of several researchers and pioneers such as Hans Krebs, George Palade, Fritiof Sjostrand, Britton Chance, and Albert Lehninger had raised the awareness on the then mysterious sub-cellular organelles.[1-3] At present called "the powerhouses" of aerobic eukaryotes, mitochondria are deemed as the center-stage for generating the major chunk of chemical energy required for performing the "works" of life. Cellular respiration is the metabolic routine that makes adenosine triphosphate (ATP; the ubiquitous storable and transportable chemical currency) and is technically called mitochondrial oxidative phosphorylation (mOxPhos). As a pre-requisite to mOxPhos, a systematic trimming and gradual stripping of electrons (or hydrogen atoms) of/from the multi-carbon reduced molecule (as exemplified by glucose, fatty/amino acids, etc) occurs to give a 2-carbon acetyl moiety. In turn, this acetyl group is taken up by mitochondrial Krebs' cycle, churning out two fully oxidized molecules of CO_2. In the overall process, some reduced molecules (like reduced nicotinamide adenine dinucleotide (NADH)/flavin adenine dinucleotide ($FADH_2$)) are formed, which are oxidized using molecular oxygen at the inner mitochondrial membrane to yield ATP within the organelle's matrix. This "oxidation of reducing equivalents using molecular oxygen, coupled with the synthesis of ATP in mitochondria" is the formal definition of mOxPhos.

The era from 1950 to 1975 saw the research community divided on the mechanism of mOxPhos, the signature metabolic activity of mitochondria. One school of thought with Edward Slater as the key exponent advocated an "enzyme-bound high-energy phosphorylating intermediate".[4] Another group led by Peter Mitchell rooted for a "proton-motive force (pmf)" driving the ATP-synthesis.[5] Long-drawn efforts to identify any enzyme-phosphorylating intermediates failed (including Paul Boyer's phased-out proposal of "phosphohistidine"),[6] and the proton-gradient hypothesis gained grounds.[7-11] Also, the chemiosmosis proposal could forge a connection between the electron transport chain (ETC, a medley of membrane-bound redox-active protein assemblies and diffusible smaller molecules) and F_oF_1ATPase (or Complex V, the "coupling factor").

Mitchell had postulated that the ETC components served to pump protons into the inter-membrane space, across the inner mitochondrial membrane. He proposed that a "proton-surplus" thus formed within the inter-membrane space leads to a pmf or trans-membrane proton potential (TMP) that drives ATP-synthesis within the matrix. Such developments inspired Paul Boyer to propose that re-entry of protons into the matrix (via the F_o module) gave the rotary synthesis of ATP by F_1 module of Complex V.[12] Some other hypotheses were also advocated: (1) Lehninger-Kasumov proposed a mechano-chemiosmotic model which posits that Complex V serves as a $Ca^{2+}/H^+–K^+Cl^-$ pump[13] and (2) Nath's torsion mechanism[14,15] posits that Complex V derives energy for phosphate-coupling by a trans-membrane anion/counter-cation gradient/exchange. These explanations also see a trans-membrane phenomenon as 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 following elements: Rotary ATP-synthesis (by Complex V),

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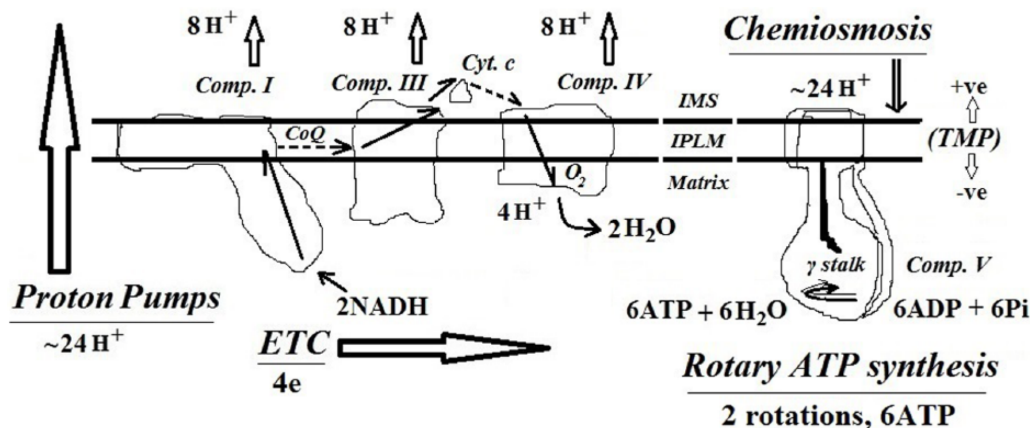


Figure 1. Cartoon representation of the salient components of mOxPhos, per the notions of the proton-centric scheme (for the Complex I ETC). The three multimeric complexes on the left side constitute the ETC, whereas the lone multimeric complex on the right constitutes the ATP-synthesizing "rotary enzyme". The diagram is not drawn to scale with respect to the dimensions of proteins or the respective positions or distribution densities. ATP: adenosine triphosphate; ETC, electron transport chain; IMS, inter-membrane space; IPLM, inner phospholipid membrane; mOxPhos, mitochondrial oxidative phosphorylation; NADH, reduced nicotinamide adenine dinucleotide.

Chemiosmosis principle working across the inner mitochondrial membrane, and Proton pumps cum Electron transport chain roles (served by Complexes I to IV). For the sake of convenience, the proton-centric hypothesis that includes the four elements above shall henceforth be called RCPE hypothesis.

Several seasoned researchers like RJP Williams, Gilbert Ling, Edward Slater, and so on continued to express concerns[14,18-23] regarding the viability of the chemiosmosis (RCPE) explanation, even after it was recognized with a Nobel Prize. Particularly, Edward "Bill" Slater (who passed away in 2016), a pioneering biochemist and the Editor of the Flagstaff journal in the field (BBA-Bioenergetics), had called upon scientists to think beyond the chemiosmosis explanation.[22] I had responded to this call in 2017 by presenting conclusive quantitative arguments to irrefutably discredit the chemiosmosis proposal and provided evidence for murburn scheme, an oxygen-centric mechanism for mOxPhos.[24,25] The newly coined term is derived from "mured (or closed) burning", connoting a mild and unrestricted redox enzyme catalysis paradigm.[24-30] The new mechanism explained physiological ATP-synthesis as an outcome of a "decentralized scheme of one-electron reactions" occurring near respiratory Complexes. In this write-up (the first part of our work), the main elements of the long-standing proton-centric (RCPE) hypothesis are summarized and critically dissected. (The murburn explanation of mOxPhos is detailed in other articles).[24,25]

The Proton-Centric RCPE Hypothesis for mOxPhos

The RCPE explanation advocates the precise orchestration of the following course of events, as given in Figure 1:

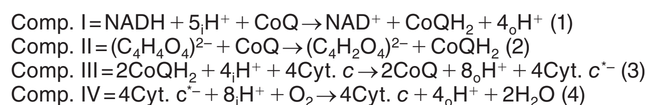
- From reduced substrates, an overall downhill relay of electrons to the electronegative oxygen molecule is achieved through a sequential and charted "chain/circuit" of large/small molecules (ETC).
- The spontaneous ETC is coupled with an uphill transmembrane (matrix to outward) proton-pumping process, occurring through three Complexes (I, III, and IV).
- The processes above set up a proton gradient across the inner mitochondrial membrane (with surplus protons in the inter-membrane space), thereby providing a chemiosmotic drive.
- Now, Complex V harnesses the chemiosmotic drive by working in a rotary mode. When the protons spontaneously move matrix-ward through the F_o module in the membrane, adenosine diphosphate (ADP) is esterified with phosphate on the F_1 domain of the complex in the matrix.

The proton-centric perspective of mOxPhos can be studied under three distinct heads: phenomenology, chemistry (stoichiometry), and electrochemical mechano-energetics. (The citations to the contents of the following section are available from the textbooks cited earlier.)

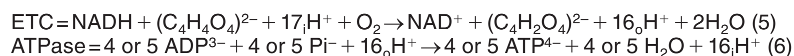
Phenomenology of the RCPE version of mOxPhos

Five multi-protein complexes, a soluble protein, and a lipophilic diffusible molecule are understood to be essentially involved in the overall process. On the inner mitochondrial membrane (toward the matrix side), electrons are stripped from NADH and succinate, to be passed on to the membrane protein Complexes I and II. Both these complexes are organized from multiple proteins and have flavins and Fe-S centers as cofactors. In turn, these complexes relay the electron-pairs on to coenzyme Q (CoQ), a series of long- or short-chain isoprenoid-quinones found freely diffusible within the inner mitochondrial membrane. Coenzyme Q can also receive electrons from other substrates like glyceraldehyde-3-phosphate (from the outside) or acyl-CoA (from the inside). All these electron transfer (ET) process are mediated via other specific flavoenzymes (dehydrogenases or oxidoreductases).

Box 1. Reaction stoichiometry of the RCPE scheme.



Comp. I = $NADH + 5_iH^+ + CoQ \rightarrow NAD^+ + CoQH_2 + 4_oH^+$ (1)
Comp. II = $(C_4H_4O_4)^{2-} + CoQ \rightarrow (C_4H_2O_4)^{2-} + CoQH_2$ (2)
Comp. III = $2CoQH_2 + 4_iH^+ + 4\text{Cyt. } c \rightarrow 2CoQ + 8_oH^+ + 4\text{Cyt. } c^{*-}$ (3)
Comp. IV = $4\text{Cyt. } c^{*-} + 8_iH^+ + O_2 \rightarrow 4\text{Cyt. } c + 4_oH^+ + 2H_2O$ (4)



ETC = $NADH + (C_4H_4O_4)^{2-} + 17_iH^+ + O_2 \rightarrow NAD^+ + (C_4H_2O_4)^{2-} + 16_oH^+ + 2H_2O$ (5)
ATPase = $4 \text{ or } 5\ ADP^{3-} + 4 \text{ or } 5\ Pi^- + 16_oH^+ \rightarrow 4 \text{ or } 5\ ATP^{4-} + 4 \text{ or } 5\ H_2O + 16_iH^+$ (6)

Coenzyme Q then relays the electrons to Complex III, a dimeric complex stationed on the inner membrane (which has hemes *b* and *c* as the major cofactors, besides an Fe-S center containing Rieske protein). Now, cytochrome *c* (Cyt. *c*, a heme *c* containing diffusible small protein present within the inter-membrane space) collects one-electron equivalents from the membrane-bound Complex III. Thereafter, Cyt. *c* shuttles across the inter-membrane aqueous microenvironment and transfers the electron to Complex IV (a heme *a* – Cu multi-protein complex, stationed on the inner membrane). All this while, the diatomic molecule of oxygen is held "bound" (tethered at the complex bimetallic center/site of heme-iron and copper) in Complex IV. This site is supposed to serve as the final "electron sink", where four electrons and four protons react or add on to the bound oxygen molecule. After the completion of this step, the dioxygen is split into two molecules of water.

It is to be noted that Complexes I through III have copious amounts of iron-sulfur proteins. All complexes (I-IV) have the oxidoreductase/dehydrogenase functionality and are also supposed to serve as proton pumps (note: Complex II does not pump protons across the membrane, but is supposed to channel protons into the membrane for CoQ to recycle). The flow of electrons is supposed to be orchestrated in real space and time via a contiguous and continuous circuitry; all in due order of increasing redox potentials: [[NADH – Flavin (Complex I) – CoQ – {Heme *b* – Heme *c* (Complex III)}– Heme a (Complex IV) – O_2]]. Except the Cyt. *c*-mediated relay, all other intermolecular ET is believed to be done through electron-pairs. However, within a given protein complex, electrons are predominantly transferred one at a time.

Finally, Complex V, a multi-protein assembly (which "senses and harnesses" the electrochemical gradient present across the membrane) serves as a chemico-mechanical motor permitting the facile entry of protons back into the matrix via its pore. This process supposedly enables an efficient synthesis of ATP, going through a cyclic catalysis strategy. After entering through *b*- and *c*-subunits, protons go through a complete rotation attached along the *c*-subunit, before gaining access into the matrix through another outlet in the F_o domain. Through one complete rotation (360°) of the γ stalk through the alternating (α-β dimer) trimers (that are held stationary by the *b*-δ subunits), three molecules of ATP are released into the matrix by the F_1 portion of Complex V.

Theoretical chemistry of the RCPE version of mOxPhos

The overall oxidation and phosphorylation reactions (for the oxidation of one molecule each of NADH and succinate, a four-electron reaction leading to the reduction of a molecule of oxygen) are captured by equations (5) and (6) of Box 1. The paradigm is essentially a two-electron scheme. As the electrons move through the Complexes I/III/IV, definite amounts of protons are supposed to be ejected (either through a proton pump or redox loop mechanism) into the inter-membrane space. The overall mass and charge balanced equation for an efficiently coupled oxygen molecule's reduction is given in Box 1.

Therefore, water is formed in two ways, via the ETC and via Complex V. Regardless of how the required four electrons are put into the ETC (through complex I or II or both), Complex IV always generates two molecules of water. (Per the mixed ETC scheme of Box 1 [for every molecule of oxygen reduced], one proton gets consumed in the matrix, and four or five molecules of water/ATP are produced at Complex V. It must be noted that a new consensus of $2H^+/2e$ is only cited for Complex IV[1-3] reaction (4) alone. In addition, later on, the consensus seemed to have evolved to three or four H^+/ATP for Complex V, which explains why four or five ATP are made through the 4e mixed scheme.) A complete rotation of Complex V uses 12 protons. (This was recently tweaked to be 9-10 protons/rotation, to address an energetics-efficiency accounting problem.) I have presented the earlier perceptions of $4H^+/2e$ scheme for Complex IV and $4H^+$/ATP for Complex V in Figure 1, as these seem to be esthetic and consistent with the original Mitchell-Boyer proposal. If Complexes I and III can pump at $4H^+/2e$, there is little reason why Complex IV cannot. Furthermore, a stoichiometry of $4H^+$/ATP fits with the trimeric nature of F_1 module that could rotate 360° through increments of 120° and also with the 12 divisions present within the *c*-subunit of F_o module. Furthermore, earlier researchers had reported that four to five protons' trans-membrane movement was correlated to a molecule of ATP synthesized. In addition, the three "sites of ATP-synthesis" demonstrated by inhibitors also support the theoretical chemistry of Figure 1. Therefore, the discussion henceforth would primarily deal with Figure 1's stoichiometry. If the reaction goes through a purely Complex I–initiated ETC (as shown in Figure 1), the reaction consumes two protons within the matrix and generates six molecules of

Box 2. RCPE equations for energetics.

$\Delta G° = \Delta G°' + 2.3\ RT \log [(C_{ADP}\ C_{Pi}\ C_{H^+})/(C_{ATP})]$
$\Delta\mu_{H+} = -F\Delta\Psi + 2.3\ RT\ (pH_P - pH_N)$
{*giving the expression* $[pmf = \Delta\Psi - 59\ (pH_P - pH_N)]$}

water/ATP at Complex V. On the other hand, if the ETC is purely based on Complex II input, protons are not consumed inside and Complex V makes four molecules of water/ATP. As evident, there is no chemico-physical connectivity whatsoever between the ETC and Complex V, other than protons pumped out (through Complexes I, III, and IV) or returning in (through Complex V). If the four electrons enter at two each through Complexes I and II, then the mixed scheme would generate five molecules each of water and ATP at Complex V.

Electrochemical mechano-energetics of the RCPE version of mOxPhos

Chemically, the combination of a set of NADH + succinate molecules gives four electrons and three protons on the inside, which supposedly leads to a unidirectional flux of ~20 protons through the inner mitochondrial membrane, into the inter-membrane space. As mentioned earlier, it was inferred that three to five protons' inward movement is correlated to the synthesis of one ATP molecule. Experimentally, it was found that in decently coupled systems, the reduction of one molecule of oxygen could give anywhere from two to six molecules of ATP, depending on the substrate oxidized. It was seen that two to four molecules of ATP for two succinate molecules as substrate and four to six molecules of ATP were yielded for two NADH molecules. Under normal cellular conditions (and with bond energetics calculation), the free energy change term for two-electron transfers from NAD^+/NADH to O_2/H_2O redox couple is approximately −220 kJ mol^{-1} and that for fumarate/succinate to O_2/H_2O redox couples is approximately −150 kJ mol^{-1}. Since the proton is a charged entity, not only the proton concentration difference affects its movement but also the presence of an electric field is also deemed instrumental. As claimed by Mitchell, the electric field arises primarily due to a charge-disparity across the membrane and is supposedly maintained by the high resistance of the inner membrane (which could be in the range of ~10^7-$10^9\ \Omega\ cm^2$). Such forces and set-ups are deemed adequate to serve as the drives for ATP-synthesis. The following theoretical equations are employed now (as shown in Box 2), to calculate the pertinent energy/force terms.

In the equations given, $\Delta G°$ is the standard Gibbs free energy change, and $\Delta G°'$ is the biological standard Gibbs free energy change (under biological conditions). C_{ADP}, C_{Pi}, C_{H^+}, and C_{ATP} are the actual concentrations of the corresponding reactants, R is the molar gas constant (8.314 J mol^{-1} K^{-1}), F is Faraday constant (96485 Coulombs mol^{-1}), and T is the temperature in Kelvin. The "P" and "N" indices denote the positively and the negatively charged sides of the coupling membrane; $\Delta\mu_{H+}$ is the trans-membrane electrochemical proton potential difference in J mol^{-1}. Proton-motive force = $-\Delta\mu_{H+}/F$ and $\Delta\Psi$ (trans-membrane electrical potential difference) are both in mV. Under biological conditions, the standard free energy change for ATP-synthesis ranges between approximately +30 and +40 kJ mol^{-1}. Rosing and Slater[31] quote the value of +33.5 kJ mol^{-1}, whereas Milo and Phillips[32] quote a number of +35 to +38 kJ mol^{-1}, under standard conditions. Therefore, a value of +35 kJ mol^{-1} can be taken as consensus. (The energy term of ATP hydrolysis/synthesis is dependent on pH, Mg^{2+}, concentration of all reactants and products. The hydrolysis energy term would become more numerically negative upon increasing ATP concentration and less numerically negative upon increasing ADP and Pi.) Since transport of a unit proton charge across the membrane is associated with an expense of ~22 kJ mol^{-1}, chemiosmosis hypothesis advocates that this expended energy is recycled when protons spontaneously return to the matrix. Chemiosmosis posits that there is sufficient scope for coupling of the downhill and uphill proton movements to bring about ATP-synthesis. Since an approximate of four protons were supposedly involved per ATP-synthesized molecule, a potential value of 88 kJ mol^{-1} would be "recyclable", which is higher than the synthetic energy term of ~35 kJ mol^{-1}. However, the energy expense (in the actual ATP-synthesis step) is deemed to be only for the release of the ATP molecule formed within the active site of the Complex V. As per Mitchell's proposal, the total of electrical cum proto-osmotic potential difference could amount to values approaching or exceeding approximately +180 to +200 mV (matrix being negative) across the inner mitochondrial membrane, and this would be sufficient to drive ATP-synthesis. Furthermore, there are at least three "sites" of ATP-synthesis "along" the ETC, as evidenced by metabolic blockers' effects. This finding is supposed to corroborate the interpretation that the Complexes I (inhibited by rotenone), III (inhibited by antimycin A), and IV (inhibited by cyanide) pump out four protons per each pair of electrons that goes into them (shown in Figure 2).

Summation of the working logic of RCPE hypothesis

The RCPE scheme (as shown in Figures 1 and 2 and Box 1) invokes a spatio-temporal separation (over significant distance/times, across distinct macroscopic phases) of protons and electrons for justifying the chemiosmosis principle. Conformational changes within membrane proteins brought about by their reduction (proffered by electrons gained from the oxidation of

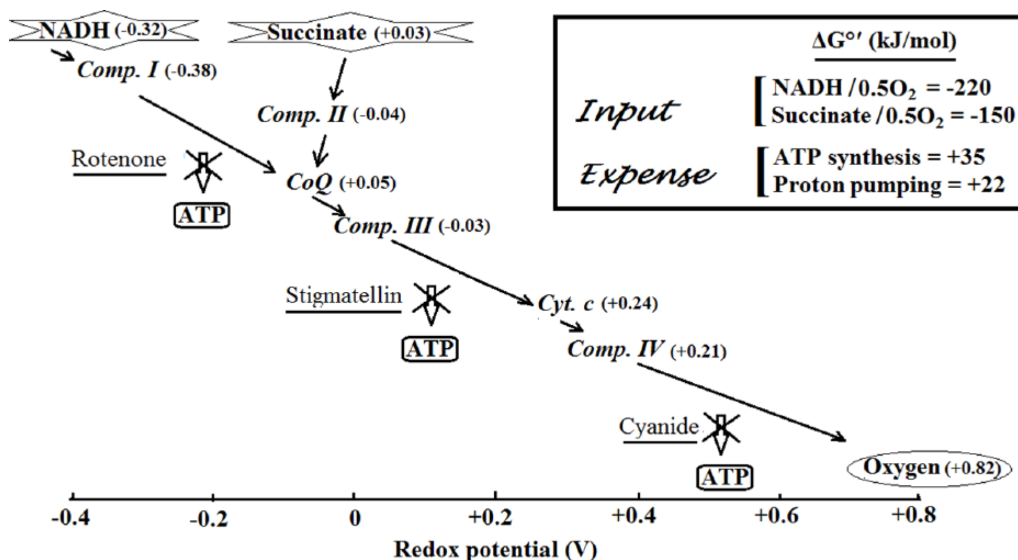


Figure 2. The energy terms of input and expenses and the distinct "energetic windows" within the ETC (along with the classical metabolic blockers of the key steps shown in underlined text). ATP, adenosine triphosphate; CoQ, coenzyme Q; NADH, reduced nicotinamide adenine dinucleotide.

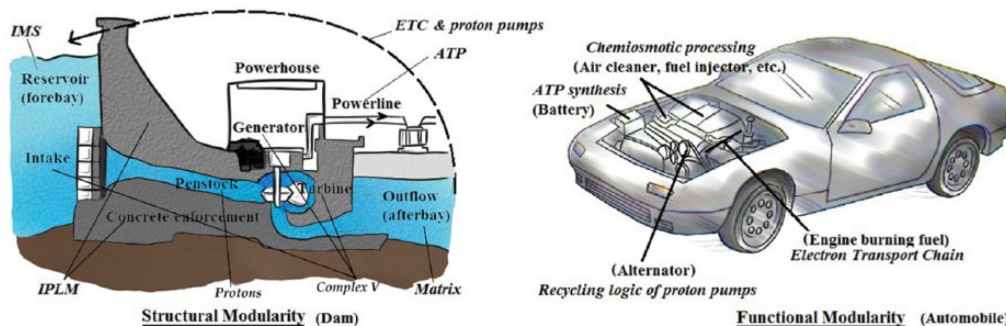


Figure 3. Pictorial renditions of working analogies of RCPE hypothesis for mOxPhos. (In both images, factual components are in normal fonts and the analogy is italicized.). ATP: adenosine triphosphate; ETC, electron transport chain; IMS, inter-membrane space; IPLM, inner phospholipid membrane; mOxPhos, mitochondrial oxidative phosphorylation; NADH, reduced nicotinamide adenine dinucleotide.

fuel molecules) serve to be the major reason for supporting proton-pumping or ATP-synthesis. Furthermore, the membrane-based electronic circuitry and proton-motive motor are supposed to work as highly synchronized distinct modules. In this scheme, diffusible/reactive oxygen species (DROS) are deemed as toxic and undesired by-products. As shown in Figure 3, the prevailing RCPE paradigm could be compared with an automobile's functional elements for energetics or a hydroelectric power plant (dam) for its structural elements (minus the "ETC + proton pumps" components). In the first analogy on the left, protons can be deemed similar to the role played by water in a hydroelectric power plant (dam). Therein, Complex V can be considered similar to a hydroelectric turbine. (This comparison lacks the structural analogs of ETC and proton pumps.) In the analogy on the right, the ability to use/capture electrochemical energy and use it for chemico-mechanical work is shown. When comparing the chemiosmotic model of mitochondria with a working man-made machine like automobile, the presence of following functional modular entities are solicited.

1. Bio-engine (ETC: burn the fuel NADH to generate the energy to do the work, retain difference of electron donating and accepting redox couples across a complex, contiguous, and compartmentalized "organic" circuitry).
2. Bio-dynamo (proton pumps: generate a proton-electrochemical gradient across the inner membrane).
3. Bio-battery (ATPsynthase: serve as the mechano-energetic coupler to cyclically synthesize the energy currency, which possesses the ability to do chemical work).
4. Bio-sensor and bio-regulator/pacemaker (chemiosmosis logic: self-analyze and self-regulate proton concentration, movements of electrons, tap into electrical field, and so on and thereafter, govern the movement/synchronization of molecular motor with pmf).

Critical Analyses of the Various Facets of RCPE Hypothesis

Herein, a forthright and critical attack is mounted on the prevailing paradigm. This is considered essential for the

advancement of science, for availing better explanations in the field, at the earliest.

Proton pumps and chemiosmosis principle

The quantitative arguments against Mitchell's proposals have already been stated in my recently published work.[24] The following is a gist of the overall arguments.

1. The most important fact is that mitochondrial matrix lacks adequate protons for any "outward pumping" kind of activity. There are only finger-countable protons ($<10^1$, at the physiological pH) in a mitochondrion, whereas there are 10^4 to 10^5 membrane-embedded protein complexes (purported proton pumps). This quantitative imbroglio is insurmountable[#A] when considering that for the reduction of a single oxygen molecule, the chemiosmosis hypothesis requires 24 protons to be pumped out (+2 more are consumed for $CoQH_2$/water formation, if NADH is the fuel). Since chemiosmosis seeks a "closed system" perspective for the build-up of TMP, it is antithetical to argue that ionic buffering or "trans-membrane proton hopping" contributes to proton replenishment. Lysis of water's O-H bond or protons from NADH cannot be sources either. Even if one concedes that differentials resulting from a miniscule amount of protons could theoretically result in a high trans-membrane potential, the charge-density would be insignificant to power the multitudes of Complex V present within each mitochondrion. Therefore, such a chemiosmotic principle[#B] or mitochondrial machinery cannot start/work from an initial state.
2. Now, even if one concedes that an adequate amount of protons are present (by some unforeseen "molecular sanctions"), the system cannot work in steady state because the function of thousands of molecular pumps cannot be synchronized. Even if one concedes that there is some inconceivable molecular governance to synchronize the pumps' activities to generate a significant TMP, the potential would be continuous, and this cannot be tapped in the mitochondrial system, which is highly discretized. A temporally staggered potential development and dissipation would be needed for the proteins to work through the "activation-relaxation" cycles of conformation changes. If Complex V is just triggered by protons or pmf/TMP alone, then the "unidirectional disposition" of the system cannot be explained. Seen in the perspective of proton dynamics, chemiosmosis proposal seeks the impossible premise that a mitochondrial matrix should have ~x amounts of protons (for proton pumps to work) and ~0.00x protons (for pmf/TMP to build) at the same instant. Therefore, the chemiosmosis system is merely an "imaginary machine", and it cannot work in steady state[#C].
3. The chemiosmosis hypothesis violates fundamental laws of thermodynamics by assuming that a dissipated force/energy is recycled through the same modality. That is, protons moving in and out of the same membrane in a closed system cannot give a viable machine for doing useful work[#D].
4. Advocates of chemiosmosis insist that mOxPhos yields only 2.5 and 1.5 molecules of ATP per molecule of NADH and succinate respectively (the so-called P/O value)[33]. This is when the cellular metabolic requirements clearly solicit significantly higher efficiencies.[24] In addition, the fact is that markedly higher yield (3.5 and 2.2 molecules of ATP per molecule of NADH and succinate respectively) have been consistently reported by many reputed groups since the 1940s[34] and emphatically asserted even in the last few decades.[34,35]
5. Furthermore, the simple/non-modularized mitochondrial architecture and membrane proteins' random distribution (and structure of proteins per se) therein do not support the operational feasibility of proton pumps or/and chemiosmosis-based build-up of TMP. Quite simply, it is highly unlikely that a few protons' movement could provide the torque for F_o module's rotation within the membrane/F_1 subunit. In this context, it is inappropriate to compare the flagellar motor (which has a very different molecular assembly) that is supposed to make a rotation using thousands of protons.
6. Table 1 presents a compilation of 2e input via complex I or II. If proton pumps worked, Complexes I, III, and IV could pump a maximum of three, one, and five protons, respectively (or a total of only nine protons) per one NADH molecule's oxidation. The corresponding RCPE consensus values are as follows: 4, 4/2, and 2/4. This quantitative disparity means that the explanation in textbooks are not thoroughly researched and/or corroborated. In addition, this means that proton accounting is totally misplaced. (The original set of explanations posits that four protons each were pumped out through the Complexes I, III, and IV; giving a total of 12 protons per 2e-traverse through ETC [from NADH]. Earlier, these 12 protons were fully required for performing one complete rotation of Complex V [to give three ATP molecules], which made some structural sense because the *c* drum was comprised of 12 subunits. Such assumptions were "conveniently changed by an authoritative consensus" [as mentioned in point (4) above] to 10 protons (with only three protons needed for 120° movement or synthesis of an ATP molecule by Complex V]. To arrive at the number 10, the earlier consensus was Complex III pumped only two protons, whereas Complexes I and IV pumped four each. Then, it was conveniently changed to Complexes I and III pumped four protons, whereas Complex IV pumped only two.)

Table 1. An overview of proton involvement and associated energetics of RCPE hypothesis for 2e input from NADH or succinate.

ELEMENT	DONOR	ACCEPTOR/ PRODUCT	H^+ DEFICIT	INPUT[a] ($KJ\,MOL^{-1}$)	PROTONS PUMPED[b]	EXPENSE ($KJ\,MOL^{-1}$)	ΔG, ENERGY DIFFERENTIAL[a] ($KJ\,MOL^{-1}$)	OVERALL VIABILITY
Comp. I	NADH	CoQ/$CoQH_2$	1	69.5	4	88	+18.5	No
Comp. II	Succinate	CoQ/$CoQH_2$	0	16.4	0	0	–16.4	Yes
Comp. III	$CoQH_2$	2Cyt. *c*/2Cyt. *c*H	0*	36.7	4/2	88/44	+51.3/+7.3	No
Comp. IV	2Cyt. *c*H	½ O_2/H_2O	**	112	2/4	44/88	–68/–24	No
In toto	NADH or succinate	NAD^+ and H_2O or fumarate and H_2O	***	220 (218) or 150 (149)	10-12 or 6-8	220-264 or 132-176	+44 (+46) or +26 (+27)	**No**

Abbreviations: CoQ, coenzyme Q; Cyt. *c*, cytochrome *c*; nicotinamide adenine dinucleotide.
[a]Numerical values in braces are calculated from the individual components.
[b]Recent consensus versus earlier suppositions.
*Needs 4e input here, in the form of $2CoQH_2$; **½ O_2 is undefined in real terms; ***Sum total of undefined operations is undefined.

Summation. The abovementioned critical analyses draw conclusive support from the well-studied systems of prokaryotes, wherein pH calculations and determinations can be made efficiently.[24] In these cellular systems (like the well-studied paradigm of *Escherichia coli*), the internal pH is chemostated, and the periplasmic pH equilibrates with the external environment. Accounting for proton numbers and pH gradients leads to the same insurmountable imbroglios (as witnessed for the eukaryotic mitochondrial system) for the proton pump–based chemiosmosis proposal. We know that *E. coli*, with a cytoplasmic pH of ~7.5, survives and grows in the gut even at pH = 9. Clearly, such bacteria should die in the scenario above, as it can no longer make ATP via proton-pumping schemes! Furthermore, it is unacceptable that RCPE seeks copious protons in the matrix for proton pumps to work, but zilch protons within for the proton gradient to form, at the same time (as would be the condition in steady state). Even if there is a miniscule proton concentration change, this cannot explain mitochondrial or cellular physiology. Finally, the mitochondrial architecture or the pertinent proteins' structures/distribution does not support any mechanism to pump protons or harness pmf/TMP.

Electron transport chain

Each element of the ETC shall be studied first and thereafter, a summation of the overall workability of the ETC is evaluated.

Complex I (or NADH dehydrogenase of bacteria). With a large trans-membrane anchoring portion and a matrix-ward projection, Complex I is a mammoth multi-protein complex with one flavin and nine Fe-S centers.[36] Figure 4 and Table 2 depict the overall structure, scheme of ET and proton-pumping facets of Complex I. The redox centers N5 and N6b were found to remain in oxidized state, even under highly optimized "reducing" conditions.[37] This could be because they are flanked by two rather "unfavorable" steps (Table 2). In addition, the Fe-S center N4 is easily accessible through solvent in both mammalian and prokaryotic systems.[25] Furthermore, two Fe-S clusters (N1a and N7) do not lie in the purported "wired" ET route.[36] Very importantly, the purported ET routes and proton pump loci are highly disconnected. In addition, as seen from Table 2, several steps of the proposed route involve unfavorable gradients/distances. From Table 1, it can be inferred that the proton pump role attributed to this protein is energetically non-viable.

Complex II. Quite like Complex I, Complex II is a multimeric protein with a single flavin, but it also a heme and has only three Fe-S clusters. The heme positioned within the trans-membrane region is not supposed to be a part of the ET route.[42] The analysis of ET within this complex is given in Table 3. As seen, the transfer step 3 is of low probability. Some researchers opine that this complex has two CoQ-binding sites, and therefore, this complex may also be involved in Q-cycle.[43] Researchers attribute two reason to the inability of Complex II to serve as trans-membrane proton pump: (1) Complex II is a relatively smaller protein complex that does not span the entirety of the phospholipid membrane and (2) the potential gradient between the donor-acceptor within this regime is too small for trans-membrane proton pumping. It is unknown why this complex alone cannot pump protons when others can.

Complex III & Q-cycle. As shown in Figure 5, the scheme for cycling of CoQ (Q-cycle, CoQ forms the junction of circuitry between the electron-equivalents input from NADH via Complex I and from succinate via Complex II, Figure 2) at Complex III is an integral component of RCPE hypothesis.[46,47] As per the RCPE scheme, the bulbous matrix-ward extension of this complex does not possess any functional relevance, and only the hemes and Fe-S center play roles in ET. The 4e Q-cycle proposal requires two fully reduced CoQ, one oxidized CoQ, two protons from the matrix side and two Cyt. *c* from the

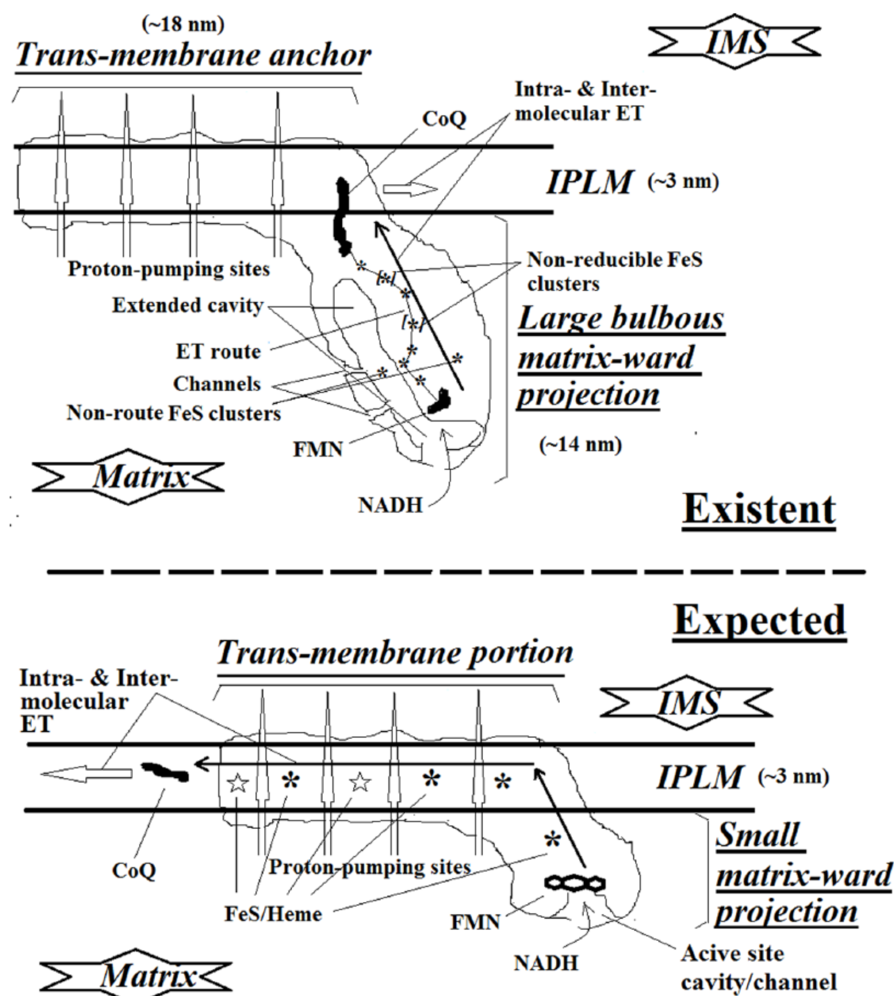


Figure 4. Top: a schematic representation of the existent ETC route and proton-pumping sites within Complex I. Clearly, the purported proton-pumping region and intra-molecular Fe-S ET have little connectivity. Bottom: the expected structure for a respiratory complex that could have potentially abided by the RCPE system. The prevailing ETC seeks a smaller matrix-ward projection (with a direct transfer of two electrons from the source to the membrane portion, without intervening solvent-accessible cavities), the burial of redox centers within TM region and specific relay of membrane-soluble redox relay agents. Then, such a series could have served as a kind of electron relay which could aid interspersed proton-pumping along the ET route. CoQ, coenzyme Q; ET, electron transfer; ETC, electron transport chain; FMN, flavin mononucleotide; IMS, inter-membrane space; IPLM, inner phospholipid membrane; mOxPhos, mitochondrial oxidative phosphorylation; NADH, nicotinamide adenine dinucleotide.

inter-membrane side (with specifically chartered movements of Fe-S Rieske protein occurring within a very precisely coordinated timescale). That is, in a single step, three different molecules (and a proton) are supposed to bind simultaneously to three different sites of Complex III. Such a multi-molecular reaction would be highly fastidious and of low probability. Figure 4 of a review by Moser et al[38] shows a snapshot of the architecture of redox centers within Complex III. The distances between the redox centers are overwhelmingly large. Mechanistic proposals like "swinging of the iron-sulfur or Rieske protein" and "electron/proton pumping and gating" are supposed to achieve the electronic circuitry's "closure".[38,48] However, there is little rationale to explain why or how the electrons flow from $CoQH_2$ back to CoQ, within the same circuit. Such a scheme would need input of power and "molecular intelligence" for realizing the "deterministic" circuitry[#E]. Docking of CoQ and $CoQH_2$ with the purported binding sites within Complex III gave inadequate discrimination, based on binding affinity or interacting amino acids.[25] Furthermore, as heme b_H is also accessible to the solvent, the necessary enzymic controls for the fastidious Q-cycle are definitely lacking. Furthermore, if the recently determined respirasome structure[49] has real physiological significance, the CoQ cycle becomes disadvantaged. This is because Complex III's trans-membrane helices dock to helices on the foot region of Complex I at one side and Complex IV at the other, limiting access to the $CoQH_2$ purportedly produced from Complexes I/II. Even otherwise, the bulky and preponderant CoQ_{10} molecules would experience significant constraints to meet the physiologically obtained ET rates (for shuttling electrons between Complexes I/II and Complex III).[25]

Complex IV. The last pit-stop of the ETC is the Cu + Fe-heme containing Complex IV. It must be noted that the overall ETC becomes defunct if the oxygen's bound presence within Complex IV is not a highly fecund and tight binding process. (It also

Table 2. Analysis of electronic circuitry within Complex I.[36–41]

STEP	DONOR (mV)	ACCEPTOR (mV)	DISTANCE (Å)	FAVORABLE ΔE*	FAVORABLE ΔD*	ELECTRON(S)/PROTON(S)
1	NADH (–320)	FMN (–340)[a]	<3*	Probable (high NADH)	Probable	2e/step (1 proton)
2	$FMNH_2$ (–340)[a]	N3 (–250 to –321); N1a (–233)	10.9 (7.6); 13.5 (12.3)	Probable; probable	Probable; less probable	1e/step (×2) (no proton)
3	N3 (–250)	N1b (–240 to –420)	14.2 (11)	Less probable	Less probable	1e/step (×2) (no proton)
4	N1b (–370)	N4 (–250 to –291)	13.9 (10.7)	Probable	Less probable	1e/step (×2) (no proton)
5*	N4 (–250)	N5 (–270 to –480); N7 (–314)	12.2 (8.5); 24.2 (20.5)	Less Probable; less probable	Probable; less probable	1e/step (×2) (no proton)
6	N5 (–430)	N6a (–250 to –325)	16.9 (14)	Probable	Less probable	1e/step (×2) (no proton)
7*	N6a (–250)	N6b (–188 to –420)	12.2 (9.4)	Less probable	Probable	1e/step (×2) (no proton)
8	N6b (–420)	N2 (–50 to –200)	14.2 (10.5)	Probable	Less probable	1e/step (×2) (no proton)
9	N2 (–150)	CoQ (–300 to –120)	11.9 (8.6)	Less probable	Probable	1e/step (×2) (2 protons)

Abbreviations: CoQ, coenzyme Q; NADH, nicotinamide adenine dinucleotide.
NADH binding was found to approach a value of $10^8\,M^{-1}s^{-1}$ (corresponding to 0.2 μs binding time). (There is a mismatch in the nomenclature of the centers, as given by Bridges et al[37] and Sazanov[36] groups. I have adopted the Sazanov numbering to cite N5 and N6b as the "non-oxidized" Fe-S clusters.]
[a]Average for 2e process; –389 and –293, respectively, for first and second e-transfer steps.

Table 3. Analysis of electron transfers within Complex II.[42-45]

STEP	DONOR (mV)	ACCEPTOR (mV)	DISTANCE (Å)	FAVORABLE ΔE*	FAVORABLE ΔD*	ELECTRON(S)/PROTONS
1	Succinate (–31)	$FADH_2$ (–79)	4.6 (2.5)	Probable	Probable	2e/step (2 protons)
2	$FADH_2$ (–79)	2Fe-2S (0)	16 (11.1)	Probable	Low probability	1e/step (×2) (no proton)
3	2Fe-2S (0)	4Fe-4S (–260)	12.4 (9.3)	Low probability	Low probability	1e/step (×2) (no proton)
4	4Fe-4S (–260)	3Fe-4S (+60)	11.9 (9.1)	Probable	Probable	1e/step (×2) (no proton)
5a	3Fe-4S (+60)	CoQ (+113)	11 (7.6)	Probable	Probable	1e/step (×2) (2 protons)
5b	3Fe-4S (+60)	Heme *b* (–185)	18.5 (11.4)	Low probability	Low probability	1e/step (×2)
6	Heme *b* (–185)	CoQ (+113)	6.5 (9.8)	Probable	Probable	1e/step (×2)

Abbreviation: CoQ: coenzyme Q.
Per literature, the turnover rate of the final step of CoQ reduction was found to be 10^2 to $10^3\,s^{-1}$.

becomes defunct if proton pumps are not operative, which is the case already established, prima facie!) Therefore, one must contend with the supposition that molecular oxygen binds to the heme-Cu center of Complex IV and stays committed to it for a protracted time frame, to sequentially receive four protons and four electrons, before dissociating as two molecules of water. It remains a conundrum why the intermediary species should not dissociate, leading to DROS formation. Evolution and deterministic outlook has its limits, a skeptic chemist would say. How can a single protein's binding mechanism evolve for holding on to the different species that would be formed? FTIR/Raman Spectroscopy experiments with several proteins' metal centers indicate that the tight-coordinating diatomic ligands (like CO and CN^-) get displaced very easily, even at low energy microcosms of –100°C. Then again, if indeed a highly versatile and evolved mechanism for oxygen

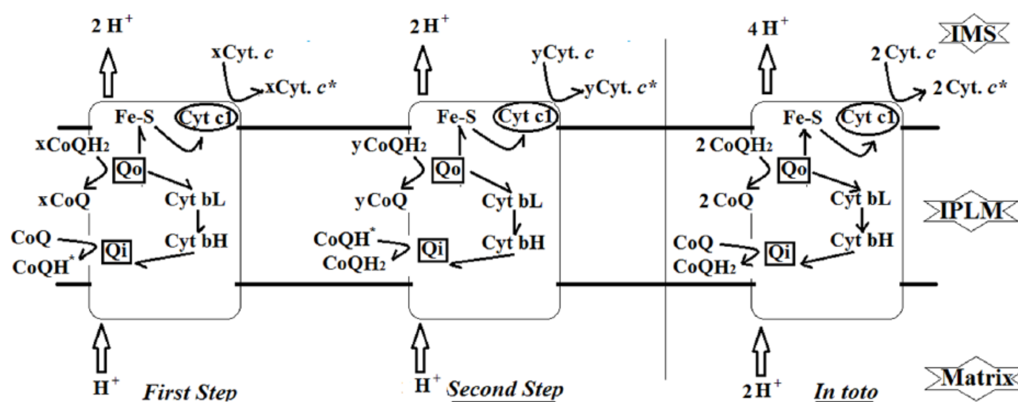


Figure 5. The various steps and circuitry of Q-cycle transpiring at Complex III. The two CoQ-binding sites are marked in square boxes and the Cyt. *c*-binding site is marked by ellipse. The x and y prefixes for CoQ and Cyt. *c* connote two different molecules (not numbers). CoQ, coenzyme Q; Cyt. *c*, IMS, inter-membrane space; IPLM, inner phospholipid membrane.

binding did exist, why do very low levels of diverse molecules and ions (a hetero-/di-atomic molecule like CO [which strongly binds to Fe(II) species] OR multi-/hetero-atomic ion like N_3^- and CN^-, which bind to Fe(III)) mess with the respiratory logic? Surely, the system could not have evolved for better binding of the toxic molecules/ions too! Repeatedly, how can the small diatomic molecule of oxygen stay bound to both Fe(II) and Fe(III) center(s) that could be spontaneously formed in the system, without detaching as incompletely reduced species (such as superoxide, peroxide, or hydroxyl radical)? Anytime during the sequence of four-electron transfers, if the tethering of oxygen failed at Complex IV, it would mean that the overall ETC must start again, and the DROS is free to attack the other redox centers within, thereby shunting ETC routes. This predicament does not afford the prevailing RCPE explanations any "workability". It is well-known that binding of a diatomic gaseous molecule like oxygen to a metal center is a temporal "on-off" (binding-detachment) process dictated by thermodynamic equilibrium. Usually, at low ligand concentrations, the amount of time the ligand is "off" is greater than the amount of time it is "on". Therefore, both quantitative and qualitative rationales dictate that a molecular tethering mechanism cannot exist for all of the different species (the combinations of diatomic oxygen ± one to four electrons ± one to four protons) that could potentially be formed on that Complex IV's reaction center. Even more interesting is to note the fact that while the K_d for oxygen was found to range between the values of 0.3 to 1 mM, the K_M value was several orders lower, at ~1 μM.[50] This is when theory dictates that $K_M \geqslant K_d$ (since $K_M = K_d + [k_2/k_1]$). Therefore, active-site binding-based kinetics treatments cannot explain this outcome. Furthermore, with a K_d value of 0.1 to 1 mM for Complex IV-O_2 interaction, the k_{off} would range from 10^4 to 10^6 per second (assuming a diffusion-limited binding value, ranging from 10^8 to 10^9 $M^{-1}s^{-1}$). It is very difficult to envisage that such an inefficient binding process would ever give an overall oxygen reduction rate of ~10^3 s^{-1} (which happens to be the physiological rate of oxygen reduction). It is also difficult to comprehend how four protons are effectively relayed deep into the protein, without affecting the bound species. In an experimental study, it was found that the rate of anaerobic intra-molecular ET between the two "heme *a*" ranged from 0.02 to 7 per second, which is several orders lower than the overall experimental ET rates.[50] This is the instance of a simple one-electron transfer step within the last leg of ETC, well within the "theoretically favorable" criteria (permissible distance and redox potential gradient)! Therefore, the "outer-sphere model" does not explain ET between redox centers within this pivotal protein.

Analysis of the overall kinetics of ETC. Since the pseudo first-order rate of substrate/oxygen depletion and ATP-synthesis approaches ~10^3 s^{-1}, it is imperative that the rate of well-coupled ET (say, for a four-electron relay from 2NADH molecules across the "circuitry" [Complex I – CoQ – Complex III – 2Cyt. *c* – Complex IV] to reduce a molecule of oxygen) must be greater than or equal to this value. This supposition for the overall water formation is supported by findings in literature.[50] It is generally agreed that transfer of a single electron between two redox centers (for the various combinations of donor-acceptor pairs: flavin→Fe-S, Fe-S↔Fe-S, Fe-S↔CoQ, Fe-S→heme, CoQ↔heme, heme↔heme, heme↔Cu, etc) across favorable potentials and permissible distances would be micro to milliseconds scales phenomena. For example, the ET within Complex I (from the FMN, all the way to the last Fe-S center, N2) was experimentally found to occur in ~10^2 μs range.[39] The maximum limit for efficient outer-sphere electron transfers in most proteins (with favorable gradients among the centers) is ~15 Å, and since the physiological proton concentration is ~10^{-7} M, the reorganization time for most proteins must fall in the range of 10^{-3} to 10^{-2} seconds (depending on the constraints protons experience to access the pertinent moiety on the protein).[51,52] The thermodynamic drive for electron flow/ETs in mitochondrial ETC scheme cannot be a "push" of electron(s) from lower to higher redox potential proteins because the arrangements of several redox centers within the ETC flout the requisites of a gradual increment of the participating elements' redox potentials. The sequential circuitry cannot be enforced through a "pull" from oxygen bound at Complex IV because the individual elements are not directly connected either. Therefore, without a significant push or pull operating,

the ET rate equation [log (k) = 12.8 − 0.6 d] applies; where k is the rate (per second) and d is the distance in Å.[38] Since the overall water formation rate has been experimentally noted to be $>10^3\,s^{-1}$, the sequential ETC scheme solicits that most transfers must be done at least at frequencies of ~$10^6\,s^{-1}$. For the same, the maximal permitted distance corresponds to ~10 to 12 Å. The circuitry components (Complexes I-IV and Cyt. c; all added up) are in the range of 10^{11} to $10^{12}\,cm^{-2}$ on/around the inner mitochondrial membrane.[53,54] For volume, let us say that this would translate to roughly several decades' μM concentration of each protein complexes, which is not a conservative estimate, by any means. Now, let us take the ETC's multi-molecular sequential reaction scheme involving at least 15 collisions/interactions of 14 participants (of which seven are distinct; NADH-2-Complex I-2-CoQ-2-Complex III-4-Cyt. c-4-Complex IV-1-O_2), each being assumed at a high concentration range of 10^{-4} M. Let us assume that even a single collision leads to a high-affinity binding and ET. Let us not forget that protons are also involved in each one of the 15 steps and they are available only at 1 M (and this is only in the bulk phase; in the trans-membrane space, it would be several orders lower). Let us forget the poor mobility of the bulky species involved and interfacial partitioning issues, and graciously assume a second-order diffusion limitation regime of 10^8 (for proteins) – 10^9 (for protons) $M^{-1}\,s^{-1}$. (Some would argue that we must invoke only two-dimensional calculations. In that case, we would also need to factor in the poor motilities for intermolecular collisions and non-availability of protons in the lipid phase. That consideration would only handicap the ETC hypothesis even more. Since electron transfers can be achieved even without intact membrane structure and since we are dealing with a system that has three-dimensional (3D) relevance, the calculation above is valid. The membrane also has a finite volume, and Cyt. c does not lie within the membrane.) A single step involving 10^{-4} M protein would maximally give a pseudo first-order rate of $10^4\,s^{-1}$, and a single step involving 10^{-7} M protons would give a pseudo first-order rate of 10^3 to $10^2\,s^{-1}$. Now, in each one of the 15 steps, these two processes are involved and let us not forget that the ETC solicits that these steps MUST occur in an ordered sequence. It makes perfect quantitative logic to argue that more than a decade of sequential steps (each with a limiting frequency of ~10^3 to 10^2 Hz) cannot work hyper-concertedly to give an overall frequency exceeding 10^3 Hz (the experimentally observed ET rates in mOxPhos). This statement becomes even more relevant considering that some reactions in the ETC scheme are two-electron or four-electron transfer steps (NADH/succinate to Complex I/II, Complex I/II to CoQ, CoQ to Complex III, and Complex IV to O_2), which would be considerably slower than the one-electron process. This deduction is furthered by the consideration that anytime a bound species dissociates without collecting the full quota of one, two, or four electrons, the circuit is broken. In conjunction with the particular issues brought out within each Complex (for example: the limiting rate of anaerobic heme-heme transfer within Complex IV), the realities (as summated above) pose insurmountable constraints on the sequential mode of transfer of four electrons within the ETC. Now, Table 4 captures the gist of overall ETC. As per the prevailing ideas, the reduction of one molecule of oxygen at Complex IV (by a total of four electrons derived from a molecule each of NADH and succinate) minimally solicits the synchronous and tandem working (or continuous linking) of ~70 proteins/small molecules present on/across the phospholipid membrane.

This ETC solicits that >24 redox-active participants (the number of one-electron redox-active species within the purported ETC) must make >54 ETs (in batches of one or two electrons) across a collective path of >600 Å (the minimal conservative distance that four electrons must travel from NADH/succinate to O_2) within the protein networks alone. If we start with NADH as the sole reductant and include a minimal distance that CoQ and Cyt. c would have to commute within the inner membrane and inter-membrane space respectively (and also factor in the distance for CoQ to recycle), we must accept that each one of the electrons must undertake a journey of $>10^3$ Å across a predominantly low dielectrics. By conservative estimates (Table 4), several steps of the proposed ETC would fall short (with respect to probability, thermodynamics, and kinetics; including the long inter-center/overall distances, number of participants and sequential/multi-molecular interactions, unfavorable gradients, etc) to explain the sub-millisecond oxidation rates experimentally observed in physiological systems. Therefore, it is safe to infer that the proposed outer-sphere sequential ETC cannot afford the overall experimental oxidation rates observed in physiology.

Conceptual notes and connectivity issues regarding the ETC concept. Besides affinity-based considerations, one-electron donating and accepting ability within the mitochondrial regime would depend on the key chemico-physical attributes of species' redox potentials, concentrations, mobility/distances, partitioning, and stability of the participating/resulting entities, and so on. Electrons do get transferred against redox potential gradients in solution chemistry, and it is viable when the relative concentrations "permit" them. That is, if the entity with a lower redox potential should receive an electron from a higher potential species, the concentration of the latter should be significantly higher than the former. Such favorable requirements can also be obviated when the donor-acceptor pairs are relatively immobilized and an external potential is applied to drive the reverse-gradient transfer. In the current mitochondrial ETC, the counter-gradient outer-sphere electron transfer would be a low probability event (within a protein) because each redox couplet or donor-acceptor pair in the "wired" sequence is at 1:1 ratio[#F].

With these fundamentals in place, the mitochondrial ETC is further analyzed at a comprehensive scale. Modularity, ordered arrangements or synchronization mechanisms are not evident in the mitochondria. Furthermore, since the membrane is a mosaic gel-fluid with a "delocalized relative positioning" of all participating components, it is very difficult to envisage a "high-fidelity circuitry" that the RCPE paradigm solicits. While NADH and succinate could transiently deliver two

Table 4. The ETC scheme for the reduction of one oxygen molecule (by the circuit of Complex I/Complex II – 2 Complex III – Complex IV)[b].

ELEMENT	REDOX PARTS	STEPS (2e + 1e)	TOTAL DISTANCE (HIGHEST) (Å)	DISTANCE PER ELECTRON (Å)	TOTAL GRADIENT (START [LOW, HIGH] END) (MV)	UNFAVORABLE STEPS	NON-"ROUTE" REDOX CENTERS
Complex I	10	1 + 16	214 (16.9, 14)	108	−320 (−480, −150) +113	14	2 Fe-S (N1a = −233 and N7 = −314)
Complex II	6	1 + 8	105 (16, 11.9)	56	−31 (−260, +60) +113	4	1 heme (−185)
Complex III	6	0 + 12 (including 6 for CoQ recycle)	[170 (34 to 20) (including 100 for CoQ recycle)] × 2	35 (not including >50 for CoQ recycle)	+113 (−90, +300) +254	6	(1 Fe-S*)*
Complex IV	6	0 + 16	120 (16)	30	+254 (+240, +320) +820	8	Nil
Overall	24	54	>750 (~10 transactions are not favored)	~230[a]	approximately −400 to +800 (~nine transactions are not favored)	32	3 (+1*)

Abbreviation: CoQ, coenzyme Q.
* Circuitry not known.
[a]This is a highly conservative estimate by any means. The value for the conservative distance of a single electron travel within "the highly efficient" supercomplex (formed by Complex I – Complex III – Complex IV) would be minimally ~350 to 400Å [as can be seen in Figure 1c of a review by Enriquez and Lenaz[55] or Figure 7b of a review by Kühlbrandt[56]]. Therefore, a multi-disrupted four-electron travel even in this "optimized" but "un-insulated discontinuously wired" system would total a distance ~1500Å.
[b]Source for Complex III distances and potentials: researchers' data[47,57]and Anthony Crofts' Cytochrome bc_1 webpage at UIUC, USA (http://www.life.illinois.edu/crofts/bc-complex_site/) and Complex III was analyzed with the ET flow starting with the step $CoQH_2$-Fe-S for both loops since the $CoQH_2$ to Cyt. b_L distance was larger than 12Å (edge to edge) and there is little reason for an ET split at the first/binding step. The distances between the respective hemes and Fe-S centers in the dimers are 21 to 63Å apart and therefore, they are not considered relevant for intermolecular ET phenomena. The data analysis is stemmed on the belief system that ubiquinone (reduced and oxidized) interact at two locale on the enzyme—the reduced species interacts via the Fe-S protein on the inter-membrane side and the oxidized species interacts via the Cyt. b_H on the matrix side. The Fe-S Rieske protein is a bifurcating point, with options to give the electron to Cyt. c_1 in the inter-membrane space or Cyt. b_L toward the matrix side of the inner membrane. The last step electron transfer timescale (to Cyt. c) is in the range of 10^0 to 10^2 µs (the fastest step of the overall process) and each of the other steps may incur a time window of 10^2 to 10^3 µs. For Complex IV, the source was a review by Moser et al[38] and the webpage—http://hyperphysics.phy-astr.gsu.edu/hbase/Chemical/redoxp.html.

electrons to the Complexes I and II systems via the bound flavin cofactors, it remains an enigma as to how CoQ receives and gives two one-electron equivalents in its interactions with Fe-S centers (in Complexes I-III, respectively). For justifying the intricate ETC within Complex III, the incoming electron-pair from $CoQH_2$ must be spontaneously split and parted to an Fe-S cluster and a heme-center. Anytime this switch does not work, the overall sequential ETC would break down. Why should Complex III route electrons through the one-electron agent of Cyt. *c*, and that too through a disconnected inter-membrane micro-aqueous phase? Surely, this would impede the ET efficiency to Complex IV. How can so many redox-active molecules and centers stay dedicated to their designated roles, without the circuitry getting shunted and without liberating DROS? It would have served well if the protein had only a few purely membrane-embedded redox centers. Why should there be so many redox centers, particularly within the matrix-protruded part of the Complex I? These facets would surely increase the probability of DROS formation in the system. How could the ETC ever function in the presence of oxygen and DROS? Why ET routes are so disconnected from the purported trans-membrane proton pumps motifs and how do these get to work in tandem? How can the passage of one-electron through a path within the intra-molecular circuit in the matrix pump two protons out? How can the ETs that occur in microsecond timescales be coupled to trans-membrane proton pumps that occur in millisecond timescales? The hitherto available RCPE concepts do not provide any answer.

Let us assume (in favor of the RCPE hypothesis) that the enzymic intermediates and intermediary mobile products ($CoQH_2$ and reduced Cyt. *c*) formed are stable entities. For the conversion of one molecule of oxygen to two molecules of water at Complex IV (via a 4e cycle), two molecules of Complex III (each working through a 4e cycle again!) must serve the four reduced Cyt. *c* substrate molecules. In turn, two Complex I/II (each of which work through a 2e cycle) would be needed to start the ETC, recruiting the required 4e from two NADH/succinate molecules. This consideration implies that the ETC components' ratio of upstream:downstream complexes must be high. That is, the RCPE hypothesis solicits a protein complex

ratio of ~two Complex I/I :one Complex IV. The mitochondrial protein distribution is quite the opposite, as Complex IV is found at much higher density than Complexes I and II combined.[53,54] (If we indulge the alternate supposition that "reactions occurring downstream are slower than upstream reactions", the imbroglio still prevails. This supposition would entail that one-electron intermediates would accrue without any thermodynamic drive for the forward reaction. Please remember that this supposition also goes against the premise that formation of water at Complex IV is the facile reaction driving the ETC!)

Inability to explain the effects of inhibitors and uncouplers on ETC. Per the proton-centric mechanism, cyanide is detrimental to ETC because it binds to Complex IV and thereby, ATP-synthesis is inhibited. The actual K_d values proffered by cyanide binding at the heme-center would be ~10^{-3} M for most heme proteins under in vitro or physiological conditions.[58] Now, cyanide has low affinities for the oxidized Complex IV, and for the reduced species, it has a comparable K_d with respect to oxygen.[59] Cyanide is a charged and asymmetric species and would be relatively less hydrophobic than oxygen. Why then, such a highly evolved hydrophobic oxygen-binding machinery of a plasma-membrane embedded Complex IV loses out even to trace quantities of cyanide (which is asymmetric and hydrophilic)? The RCPE hypothesis offers no explanation. Since a thermodynamic pull cannot be exerted from Complex IV-bound oxygen (earlier paragraph from the last section), electrons locally move across small favorable gradients via a very feeble thermodynamic push or is subjected to equilibrium within the Complex I/II – CoQ – Complex III system. Therefore, as per the RCPE hypothesis, protons should be pumped out via Complexes I and III even if Complex IV is blocked by cyanide (at least, in the first cycle). If the RCPE hypothesis were operative, an aerated and NADH replenished mitochondrial system should show some detectable ATP formation (in the presence of cyanide) because it is only the protons that sponsor Complex V activity. But the fact is that inclusion of cyanide fully inhibits ATP-synthesis within mitochondria. Therefore, the RCPE explanation fails to explain cyanide inhibition of mOxPhos.

Experimental titration of Complex I with rotenone shows at least two affinity-binding sites.[60] Rotenone is supposed to inhibit ET by binding with high affinity to the CoQ site of Complex I, near the N2 Fe-S center (of PSST/NDUFS7/NuoB subunit). Surprisingly, there is no direct evidence for this conjecture. To date, researchers have not yet been able to provide a crystal structure with rotenone bound to the purported CoQ-binding site of Complex I. With the in silico approach, we could not find any rotenone-binding cluster on the N2-containing subunit. The closest rotenone-binding site was the 255 μM K_d site (on the TYKY/NDUFS8/NuoI subunit)[25]. It is difficult to see how rotenone's lower affinity binding at such a distant site would mess the ET from N2 to CoQ. Mitchell argued that nitrophenolics (the well-known uncouplers of ETC) disrupted pmf build-up by shunting pumped-out protons back into the matrix by a flip-flop mechanism. Quite simply, this explanation cannot be deemed of any merit because nitrophenolate ion is an asymmetric large molecule with two positive and three negative charges. On the other hand, a proton would be much smaller, and it is well-known that protons have a trans-bilipid layer diffusion rate approaching 10^{-3} seconds.[61] If uncouplers worked through the trans-membrane proton shunting mechanism, brown adipose tissue's uncoupling protein's role cannot be explained.[25]

Summation. The proton-centric explanations do not offer any drive or purpose for the criss-cross movement of single or pairs of electrons (Figure 1). Super-coordinated multi-molecular sequential reactions would pose very limiting spatio-temporal dictates. Such hyper-concerted events would have little probabilities to occur spontaneously, repeatedly, and cannot add up to overall conductions within micro/millisecond time frames, particularly within the physiological concentrations of reactants, limited mobility, and spatial constraints posed by the phospholipid environment. The current ETC concept does not have any explanation for the location of redox centers outside the purported route (as seen in Complexes I/II) and cannot explain the non-reducibility of redox centers in Complex I. The "wiring" of ETC was supposed to prevent the formation of ROS, but it is seen in plenty within mitochondria. Why are multiple redox centers of unfavorable potentials separated by long distances? Why did evolution not do away with such unwanted facets that could potentially form DROS? By any thought, various junctions of this ETC might have relevance only as an ET "pit stop". For it to chug along and be operational in the physiological time frames, significant energy must be expended. Most importantly, the RCPE concept does not explain the fundamental observation of ET rate enhancement with the presentation of ADP + Pi.[24,25] On one end, the RCPE hypothesis seeks the macroscopic perspective of physical separation of protons and electrons in space and time. At the other end, it requires the very protons and electrons to be drawn together with great abundance and absolute accuracy/precision at certain defined loci/times alone. Such a scheme would have low repeatability and efficiency. In addition, if we indulge the premise that the proton pump supposition is invalid, why should electrons go through a circuitous (deterministic) route, only for reducing O_2 bound at Complex IV, to produce water? A comparative study of the prokaryotic aerobic respiratory machinery shows that the concerns relevant for mitochondrial system are relevant therein too.[25] Therefore, the prevailing ETC scheme poses little merit in esthetics, viability, and utility with respect to physiological ATP-synthesis.

Rotary ATP-synthesis by Complex V

The final element of rotary ATP-synthesis is dependent on the first three (chemiosmosis principle, proton pumps, and ETC). At the outset, let us accept that Complex V is a perfectly reversible rotary enzyme and aim to understand its function under physiological conditions.

Explaining the "forward" (ATPase) activity of Complex V. Fisher's "lock & key" hypothesis states that an enzyme has selectivity for the substrate. Koshland's "induced fit" hypothesis posits that binding of substrate can alter enzyme conformation. Both these hypotheses are supposed to lead to a "reversible transition state complex" of bound reactants or products in/on the enzyme, which has a lowered activation energy barrier than the un-catalyzed reaction complex. Thereby, the reaction can proceed more efficiently to attain equilibrium status (the direction of the reaction depending on the starting concentrations of the components). An enzyme facilitates a quicker attainment of reaction equilibrium by having greater affinities for the substrates than for the products, so that an evolutionary mandate gets served. (Otherwise, the enzyme would just continue to work in to and fro directions [at any given state of mixture], leading the system nowhere!) If the products have accumulated a lot more than the few miniscule amounts of the substrate that remains, then the products also start becoming "substrates", in spite of the low affinity that the former (product) might pose for the enzyme. In this scenario, the enzyme can (and has to!) serve in a reversible function. Summing up, an enzyme can serve reversibly when the free energy change is very low (thermodynamic drives are not major determinants) and/or the enzyme has similar affinities for the substrates and products.

Complex V is an ATPase, an ATP-hydrolyzing enzyme when isolated and assayed in vitro. This makes a lot of sense because the ΔG for this reaction is significantly negative ($-35\ kJ\ mol^{-1}$), under standard biological conditions. In normal aqueous solutions (with plenty of ATP and water molecules), thermodynamics dictate that ATP will proceed to get fully hydrolyzed and thus attain "equilibrium". That is, the equilibrium and enzyme catalysis of the equation "$ATP + H_2O \leftrightarrow ADP + Pi$" is tilted to the right. If textbooks are to be believed, Complex V's affinities are in the order ATP >>> ADP > Pi. At a given instant, let us assume that ATP, ADP, and Pi molecules/ions in the matrix are present at mM to μM concentrations. Since water is in copious amounts everywhere, we shall not bother about it. (This statement needs to be elaborated in another context. The fact that there is lots of water around is one of the main reasons why the reaction proceeds toward hydrolysis!) If we assume second-order diffusion-limited binding for all substrates and products, we can see that at a given instant, ATP has a greater probability of being found/bound on the enzyme. Since the F_1 subunit has high affinity for ATP and since water is available in plenty, ADP + Pi are spontaneously formed. Therefore, ATPase will only hydrolyze ATP even though equal amounts of ATP, ADP, and Pi are present. We had projected that in the mitochondria, the Complex V-mediated hydrolysis versus synthesis ratio (assuming the physiological parameters)[25] would approach a value of ~10^9!

Seeking to explain the "reverse" (ATPsynthase) activity of Complex V via Boyer's perspective. Let us consider achieving reversibility with a simple one-site model first. The F_o portion is supposed to transport protons at rates approaching of 10^3 to $10^4\ s^{-1}$. This corresponds to a maximal ATP-synthesis rate of ~10^3 per second, because three to four protons' movement across the F_o module are associated with the synthesis/hydrolysis of one ATP molecule. Therefore, it means that F_1 ATPsynthase must function instantaneously (with respect to the diffusion timescales) to form product(s). With micromolar level concentrations of any catalyst working on a single substrate (and graciously assuming a diffusion-limited second-order on-rate of $10^8\ M^{-1}\ s^{-1}$ for all the interacting entities), the maximal range permitted for catalysis is ~$10^2\ s^{-1}$. Now, the ATPsynthase function would need two reactants, and inorganic phosphate has very low affinities for the beta subunit. Furthermore, if protons are a reactant (particularly at slightly alkaline conditions, because the pK_a of phosphate and ADP species are around neutral pH values) or participant (as the tripping agent to gyrate the motor) in the system, the maximum catalysis rate would be far lower than the theoretical maximum quoted above. This implies that such Complex V cannot synthesize ATP in steady-state conditions (using protons) at the observed physiological steady-state rates because of limitations imposed by availability of reactants at the enzyme surface. The concept can be understood if we see the off-rates for the various entities at F_1,[25] which would be approximately $10^{-4}\ s^{-1}$ for ATP, $10^3\ s^{-1}$for ADP and $10^6\ s^{-1}$for Pi. While the catalytic rate terms are appreciable for the hydrolysis reaction (in which the enzyme has high affinity for the substrate, ATP), it is impossible to envisage how the reverse reaction of esterification/phosphorylation could ever be feasible. Qualitatively, this is explained to result because (for the esterification reaction) of the following reasons:

1. Three participants of enzyme + ADP + Pi (when considering the requirement of protons under alkaline conditions) should bind simultaneously or bind one after the other, to adjacent loci of the F_1 module.
2. The F_1 module has much lower affinities for the two substrates ADP and Pi than ATP.
3. ATP is usually found in the mitochondria at about an order higher concentration than ADP. Pi is found only in the range of its K_d value and water is available at several decades of molar concentrations.

The probabilistic point (1) above is demonstrated by a "thought-experiment", as follows. For the sake of simplicity (and for being gracious to the prevailing explanations), let us forget concentration cum affinity effects. Let us further consider the binary logic that a beta site is either free or bound by a ligand and let us consider that since there are three ligands, the probability is higher that the site is bound than it is free (3/4:1/4). When it is bound, let us consider that each one of the entities has equal probability for binding the F_1 subunit and each binding event is independent of the other. Since there is either one (when ATP alone binds) or two binding loci within a site (when ADP and Pi, both bind), the events' sample space allocation for a single binding site would be 1/4 for ATP binding, 1/16 for ADP + Pi binding, and 11/16 for other outcomes.

(Other outcomes are as follows: site remains vacant/bound to ADP only/bound to Pi only. It is not obligatory that something must be bound to each site at a given instant; particularly when the γ shaft spins around at a frequency of ~10^3 Hz and when the off-times of the participants are higher than on-times.[25]) As per the notions of the current hypothesis, the movement of γ shaft within the enzyme works as a liberator of a bound entity. Therefore, by evening out the odds stacked against the esterification reaction and considering the binding events within a single β site, ATP liberation is four times more probable than ADP + Pi liberation. This outcome does not mean that Complex V can synthesize ATP four times higher than it can hydrolyze ATP. What it means is that the probability that a pre-bound ATP molecule is released is four times greater than the probability of the release of a pre-bound ADP + Pi combination. Now, let us go beyond the concept of γ shaft being a mere releasing agent and let us also afford fully reversible catalytic function to the F_1 subunit. In this case, we get the probability that four molecules of ADP are released (from the bound ATP) when compared with one molecule of ATP released (from the bound ADP + Pi). In any straight-forward considerations, we do not have Complex V being a directional synthetic agent of ATP.

The above calculations were based on a single-site scenario. A simple extrapolation would show that if we open up to a three-sites case (or six ADP-sites as is actually the case)[25], the picture does not become better for the rotary ATP-synthesis proposal. Alternatively, let us consider for the trimeric F_1 that a molecule of ATP is bound to the first beta subunit, a combo of ADP + Pi is bound to the second beta subunit and nothing (or ADP alone or pi alone) is bound to the third beta subunit. Now, going through the cycle, at the first site, ATP is released; at the second site, ADP + Pi is released; and at the third site, the shaft just plows through without sponsoring anything. The point to note is that if the protein works as a mere adsorbing agent, it must push out the ligands. If the enzyme catalyzes, it must catalyze the forward and backward reaction equally. The movement of the γ shaft cannot wishfully work as a pushing out agent of ATP in one direction and pushing out agent of ADP + Pi in the other. (Or, stated otherwise, it cannot work as a hydrolyzer in one direction and esterifier in another.). In addition, it does not have the molecular intelligence to figure out that since it is going in a given direction, it is only supposed to synthesize. Please see that this consequence is regardless of which direction the protons flow or the shaft rotates. The conformational effects brought about in the protein would be the same, as it is an inherent spatial property of the active site. (It can also be seen in the following manner; in one way, the enzyme would cycle from high ATP affinity to low ADP affinity. In the other direction, it can still cycle only from low ADP affinity to high ATP affinity. Changing the direction does not change affinities.) If we disregard affinities, at the most, we can have a reversible Complex V afford an ATP: ADP ratio of 1:1 and not anymore! For an enzyme to preferably synthesize ATP at higher rates, it is imperative that it must have a higher affinity for ADP when compared with ATP.[25] Complex V shows the reverse case and therefore, the rotational synthesis view proposed by Boyer seems to hold little mechanistic or kinetic viability[#G].

Per Boyer, energy is required for two distinct "purposes": moving the shaft (and thereby, detaching the formed ATP still attached to the F_1 module) and getting the enzyme to have greater affinities for ADP + Pi. Boyer's first postulate is that the motor of Complex V is driven by the real-time power generated from pmf or TMP. [This was presumed to come from proton disparity/movement, which in turn, was supposedly driven by the energy derived by the oxidation of ETC fuel molecules, NADH, and succinate. Such proton-centric suppositions have already been discredited.] Energy is needed for moving the F_o's c-drum/γ-shaft, and thereby leading to the detachment of the formed/bound ATP at the F_1 module. Functionally, this is equivalent to making the enzyme lose its affinity for ATP. But then, the important question is how is the ATP formed there, in the first place? The second postulate that caters to this requisite is—yet another high-affinity site on Complex V must simultaneously bind (ADP + Pi) with a much higher efficiency than it could bind ATP. That is, in the cyclic synthesis process, the same binding sites go through a high affinity for ADP versus high affinity for ATP cycle. If Complex V functions reversibly within the system, the affinities for ADP and ATP should be quite comparable. But the in vitro/in situ works suggest that the esterification routine overcomes the rate of hydrolysis reaction and Complex V has $>10^7$ folds higher affinity for ATP (than ADP).

Regardless, let us understand the "rotary hydrolysis activity" first. In the hydrolytic function, the stalk may rotate because of the favorable ATP binding (and hydrolysis reaction). In this mode, an ATP molecule present in the matrix at high concentrations can efficiently bind F_1, get hydrolyzed and this energy can lead to a conformation change and twirl the shaft. In the meanwhile, there is a good probability that the next ATP (because it has higher affinity and is found at a higher concentration than ADP) would be bound on the adjacent site and the hydrolytic process could go on if all the other structural mandates are met. At the inter-membrane side, the F_1 activity could relay a change in the F_o domain, which could induce an inward movement of protons. (This function would not necessarily need a rotary function, and just a "lid-opening" kind of action would allow protons to spontaneously come in to neutralize the negative charges developed within.)

However, in the synthesis mode, the causative is the binding of protons on the outside. The dynamics at the F_1 subunit must ensure that both ADP and Pi bind effectively at a precise instant the proton(s) trigger the F_o module in the disconnected inter-membrane space. This seems a less probable proposition. In the best case scenario favoring the RCPE hypothesis, a

surplus of protons entering from outside through F_o module would keep rotating the γ shaft. Since the F_1 portion has high affinity for the single substrate ATP (and since ADP in matrix is significantly at lower levels than ATP), it will always outcompete the binding process of the two substrates (ADP and Pi) at the internal F_1-binding site. Therefore, the ATPsynthase could only liberate pre-synthesized ATP as the γ stalk plows through the F_1 module. Quite simply, even if the rotary facet of Complex V worked, it would be of little use for the ATPsynthase activity[#H].

The most difficult part of accepting the simultaneous synthesis cum hydrolysis by Complex V is that purportedly, the actual synthesis step of ADP and Pi coming together to form ATP does not apparently require any energy on the enzyme surface. This was inferred from physiological experiments to explain the hydrolysis:synthesis ratio of ~2.4, assuming that Complex V was the sole agent responsible for both synthesis and hydrolysis of ATP. It means that activation energy for the synthesis reaction is lowered to approach a zero value. That is, the process is supposedly (freely) reversible on the enzyme surface. If that were so, why did the cellular system go through the whole drama of ETC, proton pumps, and rotational synthesis? Homeostasis and maintenance of cellular metabolic equilibrium could have been achieved in a much simpler way without the necessity of all the complexities. In addition, if the ratio of synthesis to hydrolysis by Complex V approaches zero, we cannot have an overwhelmingly synthetic paradigm in mitochondria. Quite simply, if nature wanted Complex V to be an ATPsynthase, all that it would have required is for it to have greater affinity for ADP, compared with ATP. Research data and textbooks say that this is not the case. Complex V is demonstrated to be an ATPase, and it has several orders higher affinity for ATP than ADP. Boyer's postulates are not supported by simple evidence or straight-forward scientific deductions. It is my opinion that Boyer's proposal gathered traction only because Mitchell's chemiosmosis proposal had gained grounds and the trimeric nature of F_1 subunit and the proton channel function of F_o subunit were revealed. (When Slater's opinion was in vogue in the 1960s, let us not forget that Boyer had catered to that theory with the proposal of "phosphohistidine"[6]! Scientists do make mistakes and Nobel laureates are not infallible.[62]) The non-provable "conformation changes" and "non-harnessable" TMP cannot be quoted to serve as "marvelous tour-de-force" for overcoming the mechanistic/thermodynamic odds that the rotary ATP-synthesis hypothesis faces in physiology. No one can answer the question: how is it that trans-membrane potential difference and/or proton movements are used to increase binding affinity of the two components of ADP and Pi by several orders of magnitude (thereby, altering the very nature of the enzyme)[#I]? Very importantly, one wonders how such a "miraculous" enzyme with "irreducible complexities" evolved in the earlier time periods during the origin of life. The man-made motors and generators that employ rotary functionality have to be assembled and operated with extensive preparations, involving several vectorial and intelligent operations. And such fabricated elements do not have absolute reversibility either!

Summation. Since the ETC-based proton pumps and chemiosmosis principles have been conclusively demonstrated to be dysfunctional, the natural question that one could then ask is, what is being tapped by Complex V? Quite simply, rotary ATP-synthesis activity cannot prevail over hydrolysis with the known features of Complex V, under physiological scenarios. For reversible enzymes, one could not find even a single instance, wherein the reaction proceeds faster in the direction of lower enzyme-substrate affinity[25]. Even if we overlook the lack of protons and energetic shortcomings, Complex V or mitochondrial structure has no sophistication to afford physiological ATP-synthesis via a rotary modality. Complex V does not have any electro-magnetic induction principles or ferromagnetic components to tap into a supposed electric field that could be purportedly set up in the dynamic steady state. It could well be argued that the potential difference noted in respiring cells could be coincidental (or results owing to some reactions occurring within the mitochondrion). In addition, if a continuous trans-membrane potential (with a defined polarity of inside negative and outside positive) is what drives the Complex V, how would it afford any temporal window for any protein to go through a "native-excited-reorganize-native alternating cycle" scheme? Therefore, we can safely infer that chemiosmosis (harnessable spontaneous movement of "pumped-out protons" across the same membrane in a closed system) and rotary ATP-synthesis (Complex V changing its affinity periodically for ADP and ATP; yet affording selectivity for ATP production) proposals are "non-workable machine" logics. The only way to overcome all the cynicisms and blaring discordances in the currently prevailing explanations is to disconnect physiological ATP-synthesis from ATP hydrolysis mediated by Complex V. The fact that Complex V serves as a coupling agent in mitochondrial ATP-synthesis is definite, but it cannot be because of equilibrium-driven esterification. That is, Complex V must aid in ATP-synthesis within mitochondria by another working logic, and not as the agent directly sponsoring the phosphoester bond synthesis of ATP[#J].

Explaining the hitherto available "evidences" for RCPE hypothesis

It is now opportune to address the experimental observations which were taken to support the chemiosmosis or RCPE view of mOxPhos. (The evidences/arguments were found in the textbooks cited in the introduction section of the current write-up.)

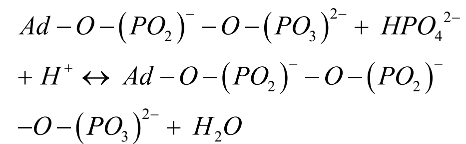
> How is the "indirect demonstration of proton pumps" explained? How are the experiments that vouched for ATP-synthesis with an initialized "proton ± ionic trans-membrane gradient" (in mitochondria/chloroplasts and reductionist models) explained?

1. When an anaerobic mitochondrial suspension with succinate + ADP + Pi was given oxygen, the bulk extra-mitochondrial pH was found to drop suddenly within seconds and return to the original value within minutes.

With a forthright deduction, this experimental outcome cannot be taken to support chemiosmosis. In fact, it should counter the "closed and coordinated system" perspective that Mitchell's postulates seek. If the "inner-membrane's proton pumps" worked, then the outer membrane is unable to contain the "surplus protons pumped out". As a result of this predicament trans-membrane potential cannot ever develop in a physiological setup. Furthermore, if the internal membrane takes the timescale of minutes to "spontaneously take the protons back in", then the physiological ATP-synthesis cannot be explained with a spontaneous inward movement of protons via Complex V. The arguments presented above can be restated with another perspective—dynamics that occur in seconds-minutes scales within an "initialized system" cannot be given as evidence for a "physiological steady state" event occurring in sub-millisecond timescales. A new explanation for the above-mentioned phenomena could be as follows. Isolated mitochondrial systems would have lowered or depleted ATP/NADH and lowered levels of Krebs' cycle metabolites. Succinate is both a Krebs' cycle metabolite and a fuel of mOxPhos. Among the Krebs' cycle intermediates, it is the dicarboxylic acid with the highest pK_a values (4.0 and 5.24, respectively, for the two acidic moieties). Input of oxygen into the milieu serves to replenish ATP levels and mitochondrial-metabolite transport systems also. This could lead to production of metabolites (with lower pK_a) leaching out and more ADP and Pi going in. Thus, these events are more likely to explain the decrease in extra-mitochondrial pH. In addition, when dyes/indicators report that the matrix becomes alkaline, it might just imply a production of hydroxide ions in the inside (owing to the hydrogen-atom deficient nature of NADH), and the outcome need not be owing to "proton-pumping activities".

2. Jagendorf's in vitro experiments showed ATP-synthesis when a chloroplast suspension pre-equilibrated at alkaline pH was exposed to an acidic buffer.

Fundamental enzyme theory dictates that Complex V could potentially synthesize ATP. This process would be viable when ATP is depleted in mitochondria (and that too, at very high levels of ADP and Pi; if we accept that Complex V's K_d value of ATP is 10^7 times lower than ADP) and when a significant external pH gradient is given. (However, this equilibrium-driven synthetic reaction would be different from the physiological steady-state ATP-synthesis which occurs without a pH gradient and at an excess of ATP.) Therefore, the Jagendorf experiment's outcome can be explained by the simple consideration that at higher pH, a closed membranous system with predominantly ADP + Pi would need protons for the synthesis of an ester bond, because the reactants' pK_a values are near neutral pH. The phosphorylation reaction in this scenario is represented by (Ad = adenosine)



$$Ad-O-(PO_2)^{-}-O-(PO_3)^{2-}+HPO_4^{2-}$$
$$+H^{+}\leftrightarrow Ad-O-(PO_2)^{-}-O-(PO_2)^{-}$$
$$-O-(PO_3)^{2-}+H_2O$$

Under these conditions, Complex V may work as an equilibrium-driven "ATPsynthase". A comparative analogy can be cited from lipase-catalyzed reactions. With normal aqueous micelles, lipolysis is favored, whereas in reverse micelles with low water content, esterification occurs.[63,64] However, in physiology, while lipase esterification is a simple reaction that can go both forward and reverse, "the interfacial machine of Complex V" cannot work in reverse under physiological conditions owing to (1) the high affinity of Complex V to ATP, (2) the synthase cycle being a multi-substrate reaction that requires ADP, Pi, and protons, and (3) particularly when the overall phenomenon involves at least three distinct phases (matrix, membrane, and IMS). The physiological reaction system for mOxPhos is faster, and it occurs at high ATP concentrations, at a range of pH values and let us not forget that protons are at a premium within the mitochondria. The fact that atractyloside and bongkrekate inhibit ATP-synthesis clearly points out that ATP-(ADP + Pi) equilibriums govern overall physiological catalysis in the mitochondria. Furthermore, in a proton-restricted environment, the thermodynamic drive for the redox reaction: $2NAD(P)H + O_2 \rightarrow 2NAD(P)^+ + 2OH^-$ goes several folds higher if external protons are available for water (O-H bond) formation. Therefore, the enhanced ATP formation with a proton gradient in an "initialized system" can be theoretically explained by multiple rationales. The most important aspect to note is that a proton gradient cannot be generated and harnessed by mitochondria in "steady state".[24] Therefore, the above experiment has little relevance to physiological realms. There is little concrete chemical or physical logic as to how the very same enzyme can reverse Complex V' affinities selectively.

3. Racker's experiment had shown that a vesicular system with rhodopsin and Complex V gave ATP-synthesis after exposure to light.

The first paragraph (second line) of the famed Racker paper[11] states that the membrane of *Halobacterium* incorporates only rhodopsin as protein. However, subsequent research (some published from the same group) had shown that membrane fractions of such bacteria include other proteins with heme-flavin systems and that such systems also showed signature DROS-mediated non-specific phosphorylations and methylations of several moieties.[65-68] In addition, there is little direct evidence (other than

perceived notions!) that ATP-synthesis in such systems resulted out of a proton pump type of activity of rhodopsin-embedded membrane preparations. The lysine-conjugated Schiff's base of the rhodopsin protein is highly alkaline pK_a (>13),[69] and it is unlikely that such a simple functional group can serve as a membrane-embedded proton pump. On the other hand, such systems are well-known to generate DROS,[70] which is expected of photo-induced transformations in the cofactor of retinal.[71] Recently, vesicles incorporating oxidase (a known DROS generator) and Complex V were also shown to support ATP-synthesis.[72] Therefore, the demonstration of ATP-synthesis by "pure" Complex V + rhodopsin vesicles can be explained with DROS chemistry, but not proton-centric pumps/potentials.

4. Employing valinomycin-K^+ with mitochondrial system for the demonstration of a chemico-protonic gradient powering ATP-synthesis or estimation of "liberated protons" is debatable. This could also negate a fundamental of Mitchell's postulates which requires an intact inner impermeable mitochondrial membrane. It is pertinent to peruse Gilbert Ling's works/writings regarding some interesting experimental observations and key conceptual ideas on cellular homeostasis.[19] Valinomycin-induced exchange of internal protons with external potassium ions remains more of an unexplained phenomenon. For example- calcium uptake by mitochondria increased with metabolic inhibitors of Complex I, III, and V; and also with valinomycin. But addition of high amounts of potassium ions lowered accumulated internal calcium. Furthermore, it was found that valinomycin did not just facilitate K^+/H^+ ion replacement and diffusion-equilibration across the membrane, but the former's concentration determined the equilibrium position of the K^+ ion in/out distribution. Using monactin and valinomycin (in conjunction with added potassium ions), it was shown that the diffusion barriers offered by the inner mitochondrial membrane (as perceived) is not owing to a continuum of phospholipid layer. It is known that mitochondria readily exchanges cations (like sodium, potassium, or calcium), has significant permeability features with respect to anions and also houses lots of aquaporin.[73] Besides the fact that the inner membrane has almost ~80% proteins, there is no direct evidence for "special features" or proton-pumping nature of the inner mitochondrial membrane. Hence, it can be argued that all types of ionic species would be subjected to several intricate networks of equilibriums within a relatively closed system. Most importantly, recent research has shown that changes in ionic equilibriums can lead to the generation of radical DROS within aqueous systems[74] and therefore, such external stimuli could induce ATP-synthesis within mitochondria. That is, if the presence of radicals within can generate chemical potential across the membrane, the presence of a potential can also generate radicals within. Thereafter, it is the radicals that bring about the useful reaction! In addition, the charge/power that can result out of ~50 nM concentration of protons (<10 protons per mitochondrion) would be insignificant with respect to the other ionic species that exist in direct equilibrium within the system[#K]. Therefore, introduction of "valinomycin-K^+"probe is not only antithetic to the hypothesis being ratified, application of the same does not allow us to trace the "cause-consequence" correlation[#L].

TMP has been observed to build-up and correlate to mitochondrial ATP-synthesis. Isn't that evidence for Mitchell's and Boyer's hypotheses?

5. Mitchell postulated a steady-state trans-membrane potential operating between the matrix and inter-membrane space of a physiologically active mitochondrion. This has not yet been demonstrated and cannot be verified in the foreseeable future because of "technical difficulty" of finding a fine probe that could be introduced into the inter-membrane space. The TMP observed,[9] which was taken as support for Mitchell's hypothesis is between bulk aqueous phase and mitochondrial matrix. Most importantly, there is no solid theoretical or structural or quantitative justification of how a trans-membrane potential could be practically transduced to give ATP-synthesis in physiological states. Elasticity-based or surface energy–based simulations/calculations (Junge's/Warschel's/Nath's works)[15-17] merely build on the prevailing "assumption" that Complex V is the ATP synthetic agent in mitochondrion that can tap a trans-membrane potential. The man-made dynamos/motors that harvest potentials via a cyclic modality employ precisely arranged components (including ferromagnetic parts) through intelligent and directional control, and the whole setup must work in a staggered modularity (with synchrony in each cycle among the dispersed individual pumps and motors). Complex V does not have any of the needed "intelligence" or structural or compositional attributes. On the other hand, it is common knowledge that the faster a motor or generator runs, the greater noise or smoke it may produce. The noise or smoke could be analogous to the generation of TMP in mitochondria. Inferring that the TMP is the causative force leading to ATP-synthesis could be erroneous. In other words, higher TMP observed with ATP-synthesis is merely coincidental, and not consequential.

Doesn't the requirement for intact mitochondrial membrane support chemiosmosis? Doesn't chemiosmosis explain the deleterious effects of ionophores, uncouplers, and so on?

6. The "non-synchronized action" of the purported proton pumps in itself should serve as an uncoupling agent, if RCPE mechanism held true.[24] Uncoupling molecules with similar structures (ie disubstituted phenolics) were also found to inhibit mXM hydroxylation system, and the mXM system does not necessitate any proton pump or intact spherical membranes.[24-30,75] In addition, phosphorylation reactions were influenced by dinitrophenol in parallel systems lacking pmf or TMP effects.[76,77] Furthermore, certain phytophenolics were also found to be deleterious for mOxPhos.[78] Thus, an uncoupling effect is observed probably due to interfacial modulation of the essential DROS dynamically generated in the system. An uncoupler like dinitrophenol would have low mobility across the highly "impermeable" membrane, particularly owing to the charge on its nitro groups. One wonders how or why they should keep dissipating an assumed gradient across the inner membrane. If it did, why is the intact mitochondrial ETC (oxygen uptake) dependent on ADP and Pi, when it is known that uncouplers/ionophores can delink ET from ATP-synthesis? Seen in another perspective, mitochondrial fragments could perform ETs, but not ATP-synthesis. How does RCPE hypothesis come to terms with the fact that intact mitochondrial systems need ADP and Pi, when the ETC does not need them at any stage? Surely, the incorporation of ionophores to determine H:P ratios would violate Mitchell's own postulates. As pointed out earlier in this writing and elsewhere, such experimental procedures and inferences must be doubted.[14,19,22] The requirement for a closed lipid membrane system could be accounted via alternative explanations. A closed vesicular environment with membrane-embedded proteins is essential to have a practically aprotic medium to stabilize DROS-dynamics and to effectively create a confined reaction zone near the membrane.

How is the recent "single-molecule" experiment with Complex V explained?

7. In recent times, studies at single-molecule level have "shown" direct rotation of Complex V.[79-82] Using a His-tag tethering of the F_1 module to a slide, the demonstration of rotation of a tagged actin filament attached to the γ shaft cannot be deemed functionally analogous to the proposed physiological functioning of ATP-synthesis. This is not just owing to the reasons quoted already in earlier sections. It must be said that single-molecule experiments have little ways of rendering spatio-temporal relevance or kinetic/energetic viability to Mitchell-Boyer's ideas. Such an experiment does not demonstrate that Complex V is "rotated" by an inward movement of protons either!

Regardless, let us try to understand the "physiological rotary functioning" of Complex V with the data made available from the single-molecule works. Let us equate the potential generated versus the power needed to turn the ATP(synth)ase, based on the information available. [To minimize complications, let us assume a mitochondrion possessing a single ATP(synth)ase.] Defining electric power as a product of voltage (0.2 V; sought by Mitchell and experimentally ratified by literature) and current (1.6×10^{-13} A; derived by assuming that about ~10 protons are present in mitochondria, and these many protons give a flux of about at 10^4 per second, $(10^1\ H^+ \times 10^4 s^{-1})/6.24 \times 10^{18}$), we have the power (=V × I) equivalent to 3.2×10^{-14} J s^{-1}. (Let us call this the left-hand term.)

From the literature on single-molecule experiments, the stepping torque (for 120°) for ATPase was determined to be ~38 pN nm, and we know that this must be generated 3×10^3 s^{-1} (because 3 × 120° = 360°; no. of rotations = 10^4 protons moving in per second/~10 protons per rotation). Since power = force × distance/time, the value of 1.14×10^{-16} J s^{-1} is the power consumption for ATP(synth)ase. (Let us call this value the right-hand term.)

Now, let us recollect that RAS hypothesis mandates that the power of the TMP roughly equates with the power for the γ shaft's movement + power for the change of conformation of the ATP(synthase) bulb. Then, calculation shows that if RCPE worked with 10 protons, only ~0.3% (of the power that was generated) is spent for moving the ATPase stalk. On the other hand, if we assume that the number of protons solicited by RCPE is somehow present in the mitochondrion (amounting to a conservative number of >100,000), then only <0.00003% is spent for moving the ATPase stalk(s). Now, where goes the rest of the energy involved/generated? (Now, if one tries to factor in the copious amounts of ATP present to the right-hand side, the left-hand term would then fall significantly short of the right-hand term!) On the other hand, if we try to lower the current component by altering the proton flux to a lower number (say, 10 protons move out/in at 10^3 or 10^2 s^{-1}), it would give still give only 3% or 30% of consumption of power generated. If we lowered the proton level to any lesser order, we would get to the unreal scenario, wherein the transmembrane potential would fail to "power" the ATPase motor. (That is, the left-hand term becomes smaller than the right-hand term.) Therefore, we must seek a particularly narrow "esthetic" proton concentration range and/or flux to justify the current component sponsored by protons. But if we do that, we cannot simultaneously meet up the demands of the experimentally observed pmf and protein densities (with the same proton/flux numbers, even if we accept a highly optimized RCPE model as discussed earlier[24])[#M].

Further, in situ, there would be several requisites for rotary ATP-synthesis. The inward movement of protons along the *c*-subunits' must twirl the membrane-embedded *c*-subunit cylinder (attached to the γ shaft) and the *c*-cylinder would also have to hold on to the membrane. The twirling cylinder must possess adequate torque to enable the γ shaft to move through the α-β dimer-trimers. Such movements in both directions must not destabilize the protein complex, and the α-β bulb must not dissociate. Furthermore, all α-β dimers should be equivalent, and so on (Clearly, since there is only one b_2 peripheral stalk and its articulation with the terminal δ hinge is asymmetric, all the α-β dimers cannot be equivalent!). Considering that the inner mitochondrial membrane has low lipid content (only 20 %), it looks unlikely that the F_o domain could find ample "fluidic-tethering" within such a "rigid membrane" (or hinge through another protein unit) and rotate at the same time. If these aspects are not comparable with the experimental setup (His-tag tethering of the F_1 module to a slide and noting the rotation of a bound actin filament to the γ shaft/ *c* drum), such an experiment cannot serve as an evidence to show that Complex V is a cyclic enzyme or a rotary synthase. Quite simply, there was little way such an experiment could have shown that Complex V is not a rotary enzyme. (It is highly opportune to cite an analogy from the mXM system here. Elaborate studies published in reputed journals had "demonstrated" several modalities/structures within an "improbable" purported catalytic cycle of cytochrome P450[83] and also a complex schema of CPR-CYP/Cyt. b_5 associations for inter-protein ET.[84] Such "unrealistic evidences" were "inapplicable" in situ, as the enzymatic system was demonstrated to recycle by more facile ways.[29,30] Therefore, physiologically relevant and chemically sound reaction models are absolutely essential to explain the mechanism of a biochemical reaction.)

Please see the predicament we are faced with—only a portion of the energy derived from NADH oxidation can be used to pump protons out. There is no way that the system can recycle the spent energy. Even if we (violate the fundamental laws of physics to) accept this proposal, only a portion of the "recycled energy" can be used. Now, the efficiency calculations of such protons' return do not match up either. Why is RCPE incapable of dealing with the ATP energetics? (The case for comparing Complex V with the "sophisticated" movement of a bacterial flagellum does not arise because the membrane structural assemblies are quite different, and thousands of protons from an open-ended source are supposed to drive the microbial flagellar system.) In the context, the reader's attention is brought to the fact that some unrealistic aspects of the rotary ATP synthetic paradigm were challenged by Berden[23] and Nath,[14,15] quoting several other reasons.

Quite simply, accounting is not possible with TMP because it is a "process coincidental variable" of the mOxPhos routine, and it is not the ultimate power source of the process outcomes. Trans-membrane proton potential is an "expression" of the ongoing reaction (power being produced/spent), and it is not the "used/usable" energy. Since we know that the ET from NADH to oxygen is physiologically linked to ATP-synthesis, the most relevant question is—when the intact mitochondria do not show oxygen consumption without ADP + Pi but disrupted mitochondria consume oxygen without being presented with ADP + Pi, doesn't that imply that the physiological mOxPhos routine is quite a different reaction system?

Bulleted case summary against RCPE hypothesis

Against a sequential ETC

Highly sequential scheme that is supposed to operate without a thermodynamic "push or pull."

A "vitally deterministic and fastidious" model that repeatedly solicits multi-molecular complexations (as exemplified by CoQ cycle).

Role allotted for oxygen (serving as a terminal electron acceptor, staying wedded to Complex IV) is rather insignificant.

The actual kinetics of physiological ET is too high, and anoxic ET rates are too low in experimental systems (that is, outer-sphere model of ETs cannot explain overall water formation rates).

Order of arrangement of redox centers of varying potentials and distances between the redox centers in/across protein complexes goes against a viable ETC.

There exists no logic as to why so many redox centers and proteins are required in the overall ETC.

Why should there be "non-route" redox centers (as exemplified in Complexes I and II)?

Why are some redox centers within Complex I not reduced (as exemplified by N5 and N6b) even in the most favorable conditions?

Natural mobility/reactivity of oxygen and other organic or inorganic molecules or protein complexes within the system must be overlooked for ETC to function (molecules need to function by "over-riding their natural dispositions").

Presence of DROS in actively respiring mitochondria and in reconstituted systems (with individual complexes) affords neither marginal utility nor esthetic appeal to ETC.

How can several synthetic dyes and redox-active molecules can put in and receive electrons from ETC (and why oxygen messes electron transport in anoxic bacteria)?

Inability to explain ET rate variations (and electron leaks) in different "metabolic states", particularly states 2 and 3.

Binding to Complex IV cannot explain the toxicity of low amounts of cyanide.

Why both low and high amounts of oxygen lead to "oxidative stress" and ROS-induced damages?

$K_d >> K_M$ conundrum of Complex IV-oxygen interaction.

Against proton pumps

Low proton availability in mitochondria (<10 protons per mitochondrion; when >100,000 are needed!) chokes the proton pump proposal.

Little direct or unambiguous structural and functional evidence for proton pumps.

Against ETC-proton pump combine

Textbooks depict that Complexes I and III each pump out four protons, which is energetically impossible (Table 1), and therefore, the ETC-proton pump cannot account for the factually observed ADP/O values for NADH and succinate.

There is no one-proton:one-electron correlation for the purported proton pumps.

The ET routes are located away from the purported trans-membrane "proton pumps."

The ETs are usually in microsecond timescales, whereas trans-membrane proton transfers are envisioned in millisecond timescales.

Against chemiosmosis

Chemiosmosis is an absolutely untenable proposal with respect to thermodynamics.

ATP-synthesis can be obtained in mitochondria incubated at pH higher than neutral values.

ATP-synthesis is noted in the absence of proton gradients and also at low trans-membrane potential.

If buffering is operational in matrix, chemiosmosis cannot work in steady state.

Steady-state perspective cannot be realized as it seeks little protons in the matrix for gradient build-up but copious amounts of protons for proton pumps to function (ie the explanation seeks the impossible realization of two mutually exclusive options).

Against ETC-rotary ATP-synthesis combine

How does blocking of Complex IV by cyanide inhibit ATP-synthesis completely in systems with higher levels of oxygen?

Against proton pump-rotary ATP-synthesis combine

How could proteins (proton pumps and ATPsynthases) change conformations in a steady state of constant unipolar trans-membrane potential (no temporal window for "native-excited-native" conformational changes)?

Against rotary ATP-synthesis

Multiple ^{18}O atoms' incorporation[76,85] into ATP.

Lack of evolutionary justification for how such a highly sophisticated rotary enzyme could be formed in the initial phases of life.

No thermodynamically compatible explanation of how Complex V could function as an enzyme that could aid ATP-synthesis within the mitochondria (when its demonstrable function is that of an ATPase).

Why does the F_1 subunit have several order higher affinities for ATP compared with ADP?

Little rationale for how Complex V taps into a trans-membrane potential in steady state.

How could the F_o subunit bind to a frail lipid membrane and rotate at the same time?

Against RCPE in toto

Non-modular organization of mitochondria with no staggered synchronization scope therein for the development of a TMP based on matrix-to-outward pumping of protons.

Relative distribution densities of mitochondrial respiratory complexes, and components do not support RCPE.

How could the operational logic of RCPE (with gambits and "irreducible complexities") evolve from a minimal set of components?

Unclear as to why Complex III would take two electrons from $CoQH_2$ and give it to two molecules of Cyt. *c* across the membrane, as this appears not just "unnecessary" but also "counter-productive" with respect to the purpose of RCPE.

Variable and non-integral stoichiometry goes against the definitive/ordered mechanism.

Maverick dose responses proffered by various molecules does not agree with specific binding-based reaction outcomes.

Requirement of ADP + Pi in the intact mitochondrial system for oxygen uptake when disrupted systems do not need ADP + Pi for oxygen uptake.

Proton-deficient NADH as the "evolutionarily chosen" molecule for the role of reductant.

Heat generation by amphipathic uncouplers and uncoupling protein of brown adipose tissue.

Why does leaching of Cyt. *c* lead to cellular apoptosis and how synthetic vesicular reconstituted systems could work without Cyt. *c* (when RCPE needs Cyt. *c* to be present in the inter-membrane space).

The favorable roles of extensive amounts of constitutive anionic lipids like cardiolipin in mitochondrial membranes.

Inhibitory roles of molecules like oligomycin and venturicidin (compared with valinomycin).

Failure to satiate a simple and facile chemical reaction logic for "coupling" and inability to meet biological structure-function correlations.

The efficiency of ATP-synthesis by RCPE is too low (1.5 for succinate and 2.5 for NADH) to account for the higher values obtained experimentally.

There seems to be little rationale for electron(s) transfers in pairs or as unit entities criss-crossing the membrane through respiratory complexes, and for physical transport from one complex to another (via CoQ and Cyt. *c*) only to form water.

Kinetically, inexplicable how hundreds of proteins/small-molecules/ions (found varying at 10^{-3}-10^{-8} M) distributed across three phases (matrix, inner membrane, and inter-membrane space) work together sequentially to make >50 ETs and channelize ~10^4 protons out/in the inner membrane via specific routes, to make 10^3 molecules of water/ATP in a second.

Conclusions

As seen from the analyses above, not only are the four components of the RCPE paradigm untenable independently, they are also incapable of forming a cohesive whole. Although there exists evidence that Complex V can synthesize ATP from an initialized state (using a proton or chemical trans-membrane potential), such findings have been explained with new rationales revealed herein. The RCPE hypothesis fails to explain the most important physiological aspect of mitochondrial metabolism—the provision of ADP and Pi enhances the utilization rate of NADH and oxygen. Since the amounts of protons are limited within the mitochondrial system, the system can only enhance its output by "speeding up its own inherent bottlenecks". This is a mechanistically incoherent imbroglio presented by the proton-centric explanation.

Very importantly, since the RCPE model is "irreducibly complex", the "evolvability" of the operating principle (Figure 3) is questioned. Each component of the perceived ETC currently serves no purpose other than to generate a "non-trappable TMP"! In addition, since the matrix to inter-membrane space proton-pumping would be an exothermic exercise (as it is funded by NADH oxidation), there is no way that the counter-directional motion of the protons across the same membrane would also be exothermic. Therefore, the inward movement of the pumped-out protons would be endothermic, and such a scheme cannot support the endothermic reaction of ATP-synthesis. Therefore, the prevailing concepts cannot explain the physiology of UCP, which is a subject of immense clinical importance.[86] In the imperative quest beyond the RCPE hypothesis, the murburn scheme (which stresses on the pivotal protagonist of oxygen and its radical products) could provide a meaningful explanation.[24,25] The insights derived thereof could help our quests in better understanding mitochondrial diseases and devising their therapy.[87]

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Author Contributions

K.M.M. conceived ideas, analyzed data and wrote the paper. Superscripted notes (#A to #M) connote optional discussions available at- www.satamjayatu.com.

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