Murburn concept: A tangible bioenergetic rationale for the origin, sustenance, termination, and evolution of life

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Abstract: The deterministic affinity-based sequential electron transport chain (ETC) of donors/acceptors and trans-membrane potential (TMP)-based chemiosmotic rotary ATP synthesis (CRAS) are the acclaimed components/principles of cellular bioenergetics. The recently floated alternative of ‘murburn concept’ advocates that diffusible reactive (oxygen) species (DRS/ROS) catalyze key physiological electron/moiety transfers. Herein, examples of organisms that disclaim the ETC-CRAS mechanism are highlighted, such as: (i) The cellular milieu of Cyanobacteria (*Prochlorococcus*) with volume of <0.1 femtoliter cannot afford free protons to pump and build TMP thereafter, precluding the functioning of chemiosmosis within this organism. Further, the anti-parallel ETCs of $\text{NADH} \rightarrow \text{H}_2\text{O}$ in respiration and $\text{H}_2\text{O} \rightarrow \text{NADH}$ in photosynthesis cannot be functional simultaneously, owing to the commonality of cytochromes b_6f and c_6 . (ii) Multi-cellular cnidarian *Henneguya salminicola* lacks respiratory Complexes I, III & IV and the unicellular protist *Plasmodium berghii* does not possess Complex V when living within blood. Since these proteins (‘proton-pumps’ and ‘rotary ATP synthase’, respectively) are obligatorily required to synthesize ATP in the classical model, the ETC-CRAS mechanism is untenable. Based on several such exceptional examples, a strong case is built against the ‘irreducibly complex’ classical explanation. Further, the agendas that validate the facile, bimolecular DRS/oxygen-based rationale of murburn concept are also presented.

Keywords: *bioenergetics; murburn concept; ATP synthesis; chemiosmosis; electron transport chain; molecular evolution*

I. The context of life and its inbuilt powering routine

Biologists believe that life originated via many favorable chanced events, from/within a cauldron of biomolecular mixture. To sustain life, living beings must also have a mechanism to catalyze reactions that are not otherwise spontaneous. For this purpose, ATP serves as the universal energy currency, aiding biological processes by tiding over unfavorable ‘thermodynamic’ premises. Ample evidence exists to support the highly probable theory that diverse forms of life we see today originated from a common ancient progenitor. Therefore, it is not convoluted to postulate that through evolution, organisms should conserve the fundamental mechanistic principle to manufacture ATP. In this regard, the powering module of any chemico-physical working system (whether a chemical engine or living organism) has the following indispensable components: food/fuel (a source of electrons), oxidant (a sink for electrons), and modalities to confine-initiate-conduct-control the reactions. The bioenergetic redox engine routine must also provide a tangible way to utilize or ‘harvest’ the redox process and ‘exhaust’ the reaction products, and continually homeostasize. Most eukaryotic aerobic living cells employ mitochondria as the cellular powering organelle (to confine-initiate-conduct-control the reactions), and use NADH as the source of electrons and oxygen as the final electron acceptor, manufacturing ATP in the process. Anaerobic cells use various electron donors and acceptors. Plant cells have the metabolic ability to respire and photosynthesize simultaneously, and they employ two distinct organelles- mitochondria and chloroplasts (respectively), for these activities.

A sophisticated proton-centric proposal floated by ‘David Keilin – Peter Mitchell – Paul Boyer’ is the acclaimed explanation for bioenergetics (Braun, 2020; Nolfi-Donagan et al., 2020). This proposal vouches that some of the ‘electron transport chain’ (ETC) proteins (Complexes I, III & IV) serve as proton pumps, leading to the establishment of ‘proton motive force’ (*pmf*) or trans-membrane potential (TMP), which in turn, enables ‘chemiosmotic rotary ATP synthesis’ (CRAS) by Complex V. Recently, an elaborate critique of the acclaimed explanation of bioenergetics was provided (Manoj, 2018a) and a theory called murburn concept was promulgated in lieu. The coinage stems from ‘mured burning’ (*‘closed oxidation’*) and implicates a stochastic scheme of equilibriums among the various reaction milieu components, i.e. molecules, unbound ions and radicals (Manoj, 2018b; Manoj, 2020a). The murburn

explanation is oxygen-centric and builds on the known principles of equilibrium-thermodynamics, reaction chemistry/kinetics, structure-function correlations and probability. This theory was built over the last two decades of experimental research on diverse heme/flavin enzymes and diffusible reactive (oxygen) species (DRS/DROS/ROS) (Manoj, 2018b; including the entire list of publications cited therein). In these aerobic/oxic reductionist systems, we showed that electron transfers and catalyses could occur outside the active sites, mediated via DROS. (In anaerobic organisms, other DRS could serve similar function.) The mechanism does not seek affinity-based complexations and works via simple collision modes. The insights derived from such studies were applied to explain the observations in various physiologies involving oxygen, such as: xenobiotic metabolism and anomalous dose responses (Manoj et al., 2016a; Parashar & Manoj, 2018; Parashar & Manoj, 2021), aerobic respiration, thermogenesis and homeostasis (Manoj et al., 2018; Manoj et al., 2019a-b; Manoj et al., 2020a; Manoj & Bazhin, 2021), photo-transduction in vision (Manoj & Jacob, 2020), oxygenic photosynthesis (Gideon et al., 2020; Manoj et al., 2020b; Manoj & Manekkathodi, 2021; Manoj et al., 2021a,b), ionic differentials and electrophysiology (Manoj & Tamagawa, 2021; Manoj et al., 2021c). In the context of aerobic respiration or oxygenic photosynthesis, the murburn model advocates that DRS produced during oxidation of NAD(P)H activates ADP/Pi and enables the direct formation of phospho-anhydride/phospho-ester. Thus, murburn model provides the first tangible chemical rationale for ‘directly coupled’ ATP synthesis in mitochondria and chloroplasts. Herein, evolutionary evidence against the ETC-CRAS proposal and the agendas that validate the murburn theory are presented.

II. Arguments against the proton-centric ETC-CRAS model

The ETC and chemiosmosis/*pmf* proposals were mooted and rooted at times when there was inadequate information on mitochondrial architecture or membrane-embedded protein structures. Our publications in recent times and the citations therein (Manoj, 2018a; Manoj et al., 2019a-b; Manoj, 2020a-b; Manoj et al., 2020a-b; Manoj & Bazhin, 2021) have brought to the fore that: (i) The proposal of chemiosmosis was floated on pure imagination and little evidence. (ii) In spite of strong opposition from leading scientists of yesteryears, the chemiosmosis idea came into vogue only because the implicit unrealistic theoretical assumptions were not identified. (iii) Several

disconnected observations were misinterpreted as supports for chemiosmosis or *pmf*. (iv) Whenever theoreticians or experimentalists discovered flaws, such aspects of chemiosmosis proposals were conveniently morphed over time. (v) There exists no consensus amongst its advocates nor is there any substantial evidence to support it even today. (vi) The structure-function correlations of the pertinent proteins and even the fundamental composition of reaction milieu do not agree with the ETC-CRAS proposal. (vii) Since researchers of the past only focused on enzyme-linked “high energy phosphorylating species” like that of phosphohistidine (Boyer, 1963), the DRS-based mechanistic eluded the scientific community. In spite of these developments, modern research pursuits in biochemistry/medicine and philosophical quests in biology are ultimately brought under the purview of chemiosmosis. Therefore, it is imperative to reorient perceptions and seek better explanations for one of the most evolutionarily pivotal aspects of life: its powering principles. To begin with, some fellow living beings are cited, to weigh in on this debate and cast their veto votes against the ETC-CRAS bioenergetic proposal.

1. With ETCs, cyanobacteria cannot respire and photosynthesize within the same cell.

Prokaryotic organisms such as cyanobacteria do not have the luxury of organellar demarcations and yet, they carry out oxygenic photosynthesis and aerobic respiration, and both processes are highly interconnected (Lea-Smith et al., 2016) and generate ATP (Figure 1). The cyanobacterial system cannot be explained within the classical perspective of bioenergetics with ideas such as: exciton relays/transfers, electron transport chains (ETC, like Z-scheme), chemiosmotic rotary ATP synthesis (CRAS), proton motive force (*pmf*), cycling proton-pumps, etc. Here is why: The purported membrane-bound ETC complexes for respiration (Complex I, Cyt. *b₆f*, and Complex IV) and photosynthesis (Photosystem II, Cyt. *b₆f*, and Photosystem I) have the common component of Cyt. *b₆f*. In the respiratory ETC, PQ (plastoquinone) receives electrons from Complex I and transports it to Cyt. *b₆f* whereas in the photosynthetic ETC, PQ receives electrons from PS II and takes it to Cyt. *b₆f*. With the ETC concept, we have a sort of ‘metabolic zugzwang’ here: electrons from the respiratory substrate of NAD(P)H can go into making the photosynthetic product of NAD(P)H. This consideration undermines the deterministic circuitry based outcomes afforded by the classical paradigm. The quinone-recycling protein Cyt. *b₆f* purportedly gives electron to Cyt. *c₆*, which could then pass it on to either Complex IV or

Photosystem I. Now, Photosystem I's electron deficient state is a highly transient one and it is unlikely that the few molecules of Cyt. c_6 present could 'voluntarily decide' to not pass on the electrons to Complex IV. Since Cyt. c_6 molecules cannot go up and down the two ETCs at the same time, NAD(P)H synthesis would become an unlikely event in daylight. Again, this is because electrons coming in from the photolysis of water from PS II would go into the downstream water-formation at Complex IV, rather than the upstream formation of NAD(P)H via PS I. Clearly, such an ETC-based metabolic scheme, which necessitates several dozens of electron transport steps (among ~20 odd redox-active participants) for the formation of a simple molecule like NAD(P)H and cannot prevent the futile drainage of redox power in the respiratory-photosynthetic cycles can't and won't evolve spontaneously! Recent publication from our group have shown new mechanistic routes for electron transfers in such systems which necessitate us to outgrow the concepts such as ETC (Gideon et al., 2020, Gideon et al., 2021).

Figure 1: Left panel- A simple schematic of the anti-parallelism of respiration and photosynthesis in the cell of a cyanobacterium like *Prochlorococcus*. While both processes produce ATP and generate heat (not shown), respiration consumes oxygen whereas photosynthesis liberates oxygen. **Right panel-** The electrochemistry of cyanobacterial thylakoid membranes (in yellow) with the ETC of photosynthesis and respiration is shown in the left and compared to a simple electrochemical setup on the right. The direction of proton pumps is shown in white arrows and the direction of electron transport across various phases is shown in line arrows. The ETC and proton pump loops seek the impossible premise of steady state movement of electrons in haphazard manner with respect to the potential at one hand, and movement of protons too in bidirectional ways across the same membrane! Further, while electrons rise in the cytoplasm and sink therein itself within the respiratory ETC, the photo-electrons are sourced from lumen and sunk in cytoplasm. Quite simply, these loops and routes violate all known concepts of electrochemistry and working logic of chemical systems.

Very importantly, the ETCs and proton pumps working logic is compared in the right panel of Figure 1. As seen, the Mitchellian proposals violate fundamental concepts of electrochemistry and feasibility for getting a working/workable chemical system. There is no evidence to say that the ETCs of the two anti-parallel metabolic schemes are arranged distinctly or separately in the

cyanobacterial thylakoid membranes, in the necessary sequence and orientations. There is no evidence whatsoever that recycling proton pumps could work in such a system, as the structure of *Prochlorococcus* merely shows the respiratory/photosynthetic pigments strewn around on the thylakoid membranes (Partensky et al., 1999). *Prochlorococcus* is a coccoid (sphero-cylinder) photosynthetic organism, of approximately 0.6 x 0.4 x 0.4 micron dimensions. The amount of free (dissociated) protons in any such a cell would be = (volume of cell) x (concentration of protons) x (Avogadro's number) = $(0.085 \times 10^{-15}) \times (10^{-8}) \times (6.023 \times 10^{23}) = \sim 0.5!$ Within a cyanobacterium, the number of photo-respiratory proteins would be $\sim 10^5$ and the cycling proton pump concept would require at least two orders excess of this amount for efficient chemiosmotic cycling. Therefore, the concept of cycling proton-pumps and proton motive force (*pmf*) fails, negating the ETC-CRAS proposal, and wishing for such systems to violate laws of thermodynamics (Bal et al., 2012) is just vain apologetic fervor. In stark contrast, murburn bioenergetic model recruits free electrons and diffusible reactive species (DRS), which can explain the cyanobacterial system well.

2. Some aphids can respire and photosynthesize without complex setups such as Z-scheme.

Alain Robichon's group reported the light-induced electron transfer and ATP synthesis in a carotene-synthesizing insect, *Acyrtosiphon pisum* (Valmalette et al., 2012). These animals perhaps acquired the gene for carotene synthesis from plants via horizontal/lateral gene transfer. However, they do not have photosystems and chlorophyll binding proteins or light harvesting complexes arranged to function in elaborate ETC, such as the Z-scheme. In the insect, carotenoids are found either distributed freely in hemolymph (attached to hydrophobic patches of proteins) or bound to hydrophobic membranes of cells. In the classical view, carotenoids have only photon-harvesting or exciton transfer roles in photosynthesis. In the murburn model of photo-transduction, carotenoids can give out electrons and replenish them from various sources in the photosynthetic cycle (Manoj & Jacob, 2020; Manoj & Manekkathodi, 2021). The delocalized nature of NADH and ATP synthesis advocated by murburn model can explain the outcomes in the aphid system whereas the classical model falls flat. The outcome observed in aphids is akin to the observations of enhanced ATP-synthesis with low-level laser therapy (LLLT) in humans. LLLT is associated with increased diffusible reactive oxygen species

(DROS) observed in these systems (Freitas & Hamblin, 2016) and it was also shown that the photomodulation's primary target is the mitochondrial water pool (Sommer, 2019). As per the murburn model, upon photo-excitations, suitable chromophores (like hemes, flavins, melatonins, etc. in mitochondria) of human cells can give out electrons (which gives rise to DROS). These DRS, in turn, can directly aid ATP synthesis and the electron deficiency can be replenished from other sources thereafter. This murburn perspective explains the overall observations.

3. The chemiosmosis perspective suggests distinct bioenergetic origins for archaea.

Along with the cell/protoplasm theory (cell or protoplasm is the basic unit composition of life – we don't consider viruses and prions as living beings!) and central dogma (genes code for proteins and these two govern key perpetuating attributes of a living species), the bioenergetic principle (chemiosmosis or murburn concept?) would form the three pillars of life on earth. In this regard, it is interesting to note that archaea have very distinct bioenergetic membrane-machinery. While bacteria/eukarya have fatty acyl chains esterified to glycerol-3-phosphate, archaea have isoprenoid chains etherified to glycerol-1-phosphate. While bacteria/eukarya have ubiquinone/plastoquinone with unsaturated isoprenyl chains, archaea have methazophenazines or menaquinone or thiophene bearing quinones with saturated alkyl chains. The enzymatic machinery for making these components is distinct and not connected/conserved (Sojo et al., 2014). Archaea like *Ferroplasma* have a bolamphiphilic monolayer (Macalady et al., 2004) and only a single bi-layer is present in bacteria (wherein the periplasm is in equilibrium with the environment). These facts are incompatible with the ETC-CRAS requirement of two bilayered membranes. Although Mitchellian postulates seek that both mitochondrial membranes present in eukarya must be impermeable to protons, the reality does not meet the expectations. Further, it is also known that the periplasm of prokaryotes equilibrates with the external environment and thus, cannot support chemiosmosis (Manoj et al., 2019a). While it is believed that bacteria/eukarya possess a conserved F_0F_1 ATP(synth)ase, archaea lack the genes completely and have a significantly different V-ATP(synth)ase. The structures of these two protein complexes are significantly different and it is evident that they must work very differently. Researchers have tried to reason out such grass-root level dichotomy with LUCA (Last Universally Common Ancestor) within the purview of chemiosmosis (Sojo et al., 2014). This is an impossible errand,

as the proposal of chemiosmosis is a thermodynamically unviable concept, which has no parallels in the physical world outside the bioenergetic community dealing with mitochondria/chloroplasts.

Archaea are known to inhabit a wide range of habitats: some thermophilic species of *Methanopyrus* can reproduce at 122 °C, halophilic species of *Halobacterium* can grow at NaCl concentrations of 25%, alkaliphilic species of *Natronomonas* can thrive at pH values of 11 and acidiphilic species belonging to *Picrophilus* can live at pH 0. Under the chemiosmosis purview, the key players are “smart membranes/proteins” and they must function in cooperative unison across these diverse habitats to maintain pumps and gradients of H^+ (and/or Na^+ , as this ion also seems to have come under the purview of chemiosmosis now). Any skeptic would question this premise because protons are known to have critical equilibria with various components in milieu and proteins can easily change their attributes in such variable ambiances. The mitochondrial system is finicky, which changes its metabolic profiles with a few folds variations of ionic strength, pH and temperature. Also, it is just a perception that biological membranes (like that of mitochondria/chloroplast) are impermeable to protons. If it were, the Jagendorf experiment (demonstration of ATP synthesis with an external pH gradient: erroneously taken as support for Mitchell’s hypothesis!) would not have given any results! We know that protons cross phospholipid membranes in millisecond time-frames (Biegel & Gould, 1981). Most importantly- chemiosmosis requires the maintenance of events in three disconnected phases for it to be operative; and it is not perceivable with any stretch of imagination how the archaean habitats and infrastructure would permit this seemingly impossible mandate. It is easy to see with the murburn model that organisms can recruit ‘various molecules – unbound ions – reactive species’ interactive equilibria for harnessing energy, across the diverse habitats.

4. Some proteobacteria show violation or irrelevance of ETC-CRAS mechanism.

The genes of ATP(synth)ase are considered obligatory and conserved in bacteria/eukarya. However, the genes of this protein complex are located clustered at two different/distant loci in *Rickettsiae prowazekii*, which signifies its low evolutionary significance for the organism (Andersson, 1998).

In aphids, the proteobacterial strains of *Buchnera aphidicola* reside as endo-symbionts in specialized gut cells called bacteriocytes. A *Buchnera* strain isolated from the aphid *Cinara cedri* showed the absence of all the eight genes of F₀F₁ATP(synth)ase (Charles et al., 2011). It is difficult to think that the aphids-bacteria symbiotic association dating back more than a hundred million years could be viable for the two species if one of them was an energy parasite over the other. Also, it is difficult to see how these bacteria could take in ATP from its host when they have three membranes separating them, have little ATP-dependent transporters and their cells are present in several layers (Perez-Brocal et al., 2006). These findings suggest that the bacterium must have some efficient ways to synthesize ATP, that is independent of F₀F₁ATP(synth)ase.

Proteobacteria comprise of a wide variety of organisms with an equally wide metabolic repertoire, and they are also found as extremophiles, with respect to temperature, pH, salinity and nutrient requirements (quite like the archaeans). They could be chemolithotrophs, phototrophs, heterotrophs, etc. or aerobic and anaerobic or free-living and parasitic/pathogenic, etc. While we can consider the archaeans' unique structural components to aid their adaptability to extreme environments, the proteobacteria have only the usual biochemical options available to help them in the extreme environments.

The metabolic diversity of one campylobacterium species is presented here, with respect to the nature of electron acceptors usable in the respiratory reaction. *Sulfosprillum multivorans* (van der Stel & Wosten, 2019) can use a wide array of species like: neutral diatomic gas like oxygen; organic anion like fumarate; halocarbons like perchloroethene and trichloroethene; neutral or charged organics like dimethyl sulfoxide and trimethylamine oxide; inorganic anions like perchlorate, nitrate, nitrite, tetrathionate, arsenate, selenate, thiosulfate, etc. These species have a wide range of redox potentials, have various molecular dimensions/geometries, topologies, bond-rotation abilities and electrostatics/H-bonding, partitioning effects, etc. How could such diverse species be bound at the active site of the protein to relay multiple electrons to it? (The mandate of passing on four electrons to molecular oxygen to make two molecules of water is referred here! Can one imagine the same fate for all these molecules, with the same protein or with new protein for each case?) Also, it is impossible to imagine that such an organism can accept

electrons from a wide range of anionic or neutral organics (like formate, pyruvate, lactate, ethanol, glycerol, etc) and inorganics (gas like H₂S or ion like sulfide, etc.) through some specific affinity based mechanisms and pass it on to the diverse final electron-acceptors through the affinity-based mechanism yet again..... all this, only to deterministically relay the electrons through an elaborate scheme of ETC to generate a non-harvestable and non-existent pH gradient! While the ETC-CRAS proposal cannot explain the diversity of metabolic repertoire, the chemical reactivity based rationale of murburn concept (which does not necessitate a binding-based recognition of a protein's active site) accommodates the versatility.

5. Some eukaryotic protists lack parts/whole of F₀F₁ATPase/mitochondria altogether.

Several ciliated pathogens belonging to the Apicomplexa and one strain of free living oxymonad *Tetrahymena thermophila* were found to lack key components of the F₀ module of the Complex V (Balabhaskaran Nina et al., 2010). *Plasmodium berghei* is a malarial parasite in rodents which does not possess Complex V in the blood-stage of its life cycle (Sturm et al., 2015). Although several eukaryotes were known with mitochondria related organelles (MROs), the first true microbe lacking any mitochondria or MROs was first reported in *Monocercomonoides* (a tetra-flagellated protist found in respiratory tract of insects and vertebrates) few years ago (Karnkowska et al., 2016). Therefore, there are several examples of single-celled eukaryotic systems that show ATP synthesis without the essential components of ETC-CRAS mechanism. In the murburn model (Manoj et al., 2019a-b), Complex V is not the ATP-synthase and therefore, these reports are accommodated.

6. The multicellular cnidarian *Henneguya* lacks the 'ETC/proton-pumps' of chemiosmosis.

Recently, it was reported that the multicellular cnidarian parasite of salmon (living in low-oxygen conditions), *Henneguya salminicola*, does not possess mitochondrial Complexes I, III & IV (Yahalomi et al., 2020). These are the purported proton pumps of the ETC-CRAS proposal. Since murburn concept can explain ATP synthesis even with Complex II (which is not believed to enable trans-membrane proton pumping) and generate DROS with other heme/flavin proteins of the milieu, the ATP synthesis in this multi-cellular organism can be explained. Since Complex

II is not considered to be a proton pump, the physiological functioning of Complex V is rendered redundant in this organism, thereby annulling the ETC-CRAS proposal.

III. Comparative summation on the evolutionary/physiological relevance of the models

Besides the above quoted naturally occurring life forms, a plethora of mutation and knock-out studies carried out in various prokaryotic and eukaryotic experimental models show results that cannot be accommodated within the ‘irreducibly complex’ (an anti-evolutionary term coined by Michael Behe) ETC-CRAS proposal. For example: *E.coli* with mutated and dysfunctional Complex V survived (Klionsky et al., 1984). Complex I deficient mutants survive in *Arabidopsis thaliana* (Fromm et al., 2016) and the European mistletoe (*Viscum album*) lacks Complex I altogether (Senkler et al., 2018). Mutants lacking plastocyanin and cytochromes *c₅₅₃/f* can live almost like the wildtype plants/cyanobacteria (Zhang et al., 1994; Fernandez-Velasco et al., 2001; Pesaresi et al., 2009). Therefore, the ETC-CRAS proposal fails the definition and mandate for a universal/ubiquitous bioenergetic routine logic (as detailed in the introductory paragraph). Proton-gradients that could give any meaningful rightward tilt to the equilibrium ($\text{ADP} + \text{Pi} + \text{H}^+ \rightarrow \text{ATP} + \text{H}_2\text{O}$) don’t exist in physiology and therefore, chemiosmosis is a mirage. The classical active site binding mechanism fails to explain the small lethal dosage or acute toxicity of cyanide. The chemiosmosis model does not provide any viable exhaust logic or explanations for thermogenesis/homeostasis whereas the murburn model is comprehensive in its package (Manoj/Manoj et al., 2018-2021). It does not make sense evolutionarily that substrate phosphorylation seems to have higher efficiency than the classical model for oxidative- or photo-phosphorylations; and yet, nature chose the latter over former. The evolutionary significance of DROS in plant physiology is underlined by the fact that leaves showing high rates of photosynthesis shed/die quicker than the rest (Matsuki & Koike, 2006). Further, molecules like NADH and ATP would be needed quickly and at high demand in cells, which the chloroplasts are mandated to furnish. It does not make evolutionary purpose that a simple hydride transfer to NADP^+ or phosphoryl transfer to ADP would require several dozens of electron transfer steps, as mandated by the ETC concept. One would expect evolution to work in the reverse way: to simplify the bioenergetic chemical reactions, and not to increase the steps and components! The reaction logic of murburn model of bioenergetics requires only: (a) a molecule/ion (like NADH

or sulfide) with low redox potential, serving as a source of electrons + (b) a *d*-orbital/ π -electronic system to serve as catalyst (like heme or flavin) + (c) a molecule/ion (like O₂ or Fe³⁺) that can accept electrons. The ETC-CRAS proposal requires not only the above items (a) through (c), but also: (i) several sequentially acting electron donor/acceptor proteins/molecules embedded in a closed phospholipid membrane, which is in turn enclosed in yet another membrane + (ii) electron transfer/transport catalysts working in intricately woven deterministic pathways across three different phases, recognizing each other with topological affinity + (iii) proteins that can couple electron transfer to trans-membrane proton pumping and TMP generation + (iv) the mutually exclusive options of ‘high concentration of protons in a given phase’ to serve proton pumps and ‘low protons in the same phase’ for generating TMP + (v) sophisticated membrane-embedded molecular motors triggered by protons/TMP + (vi) intelligent membranes that regulates the in/out flux of several components including protons + (vii) molecules behaving in ways alien to their inherent nature (oxygen must bind only at Complex IV and remain bound until it receives four protons and four electrons, Complex V must work as an ATPsynthase against the mandate of equilibrium and its own nature-destined disposition as an ATPase, etc.), (viii) the overall system must violate the laws of thermodynamics and generate energy from endergonic processes, etc. Therefore, the murburn model makes the Okham’s razor cut. While Michael Behe called it correctly that the ETC-CRAS mechanism is irreducibly complex and cannot evolve spontaneously, the realities do not mandate that there is any ‘deterministic creative hands working behind the scenes’ either. To my knowledge, the known structural aspects on mitochondria and its components fit well within the statistical reaction mechanism offered by the stochastic murburn paradigm, which gels well with the consideration that life evolved from chaos. While murburn concept’s principles are ubiquitous and well-grounded, the ideas of chemiosmosis, ETC, proton motive force, rotary ATP synthesis, etc. violate fundamental notions of chemico-physics and are unheard of in any realms other than bioenergetics. A comparison of the two models is made in Figure 2. The structure-function correlations of the various mitochondrial complexes are given in Figure 3 and that of chloroplast complexes are given in Figure 4. The ETC-CRAS model cannot justify the wayward locations of several redox centers of inappropriate redox potentials. Also, the large bulky protrusions with/without redox centers and multiple ADP binding sites are inexplicable in its purview. The ETC-CRAS mechanism is analogous to a man-made hydroelectric power generation system or automobile whereas the

murburn model functions similar to a spontaneously formed hearth or nuclear reactor (Manoj, 2018a-b). DRS mediated formation of peroxide in the murburn scheme allows the synthesis of ATP at mM levels (Manoj et al., 2019b), which, in turn, enables ATP-hydrolysis mediated endergonic physiologies. In this context, it is imperative to register that a steady trans-membrane potential (TMP) is not the directive/causative principle of oxidative-/photo- phosphorylation, it is the consequence of the same. In the era before the popularization of ETC-CRAS mechanism, researchers had explored other modalities of phosphorylations in prebiotic systems (Schwartz & Ponnamperna, 1968). It is natural to imagine that murburn mechanisms would have been prevalent then too, as it is even today in biospheres (for example: the degradation of lignocellulosics and recycling of halogenated organics) (Manoj, 2018b). Also, the formation of peroxide is not a mere wasteful/toxic reaction (as deemed by the classical perspective), as it can further be recycled for peroxidative phosphorylations. Contrary to popular perceptions, recent research has unraveled that peroxide formation is facile from water (Lee et al., 2019) and it can spontaneously exist in equilibrium with superoxide and hydroxyl radicals in water (Stoin et al., 2013). Further, peroxide is now also recognized as a key molecular messenger (Di Marzo et al., 2018; Rashdan & Pattilo, 2020; Tena, 2020). Therefore, murburn model offers a tangible and comprehensive chemico-physical explanation with respect to the bioenergetics of life.

Figure 2: A comparison of the proton/TMP-centric ETC-CRAS and the oxygen/DRS-centric murburn model for bioenergetic phosphorylations. On the left is shown the ETC-CRAS elements, which includes a closed cycling of protons to build TMP and harness the same via the synthesis of ATP at Complex V. There is no direct chemical connectivity between the electron transfers or TMP formation or ATP-synthesis. On the right is the essential features of murburn model, which involves the activation of oxygen via redox center on the proteins (denoted by star, on Complexes I through IV), which enable effective charge separation (ECS) and substrate presentation (ADP bound on the protein). The DRS produced in oxidation of redox equivalents directly activates ADP/Pi, and thus leads to the formation of peroxide in milieu, along with ATP and water. In this model, Complex V serves as pH/ion-chemostat. Formation of radical species (hydrogen atom, superoxide, hydroxyl, etc.) would be facile and they could directly mediate the coupling of the oxidative process with the phosphorylation group transfer activation chemistry.

Figure 3: Structure-function correlations of mitochondrial membrane-embedded redox proteins, under ETC-CRAS and murburn purviews. In the ETC-CRAS model, the protein complexes only serve to pump protons and their large matrix-ward protrusions are not attributed useful functions. Clearly, the ET path (depicted with dashed lines) and proton-pumping routes (shown as white arrows) are disconnected. Several steps are multi-molecular, requiring affinity binding of multiple substrates (shown with black arrows) for protracted time frames. There is no chemical coupling between substrate oxidation and ATP synthesis. In stark contrast, the large protrusions within the matrix have functional roles in the murburn model, as they bind ADP and present it efficiently for DR(O)S/activated phosphate attack around the vicinity of the complexes. The murburn model allows direct bimolecular coupling of substrate oxidation with phosphorylation of ADP (not shown here, but the schematic is visualized in Figure 2). The ETC-CRAS is deterministic and predominantly 2e scheme, whereas the murburn model is stochastic and primarily 1e reaction logic. While oxygen freely reacts with all Complexes in the murburn model, it is supposed to stay (inexplicably!) bound to Complex IV in the ETC scheme, and serves only to make water. CoQ and Cyt. c need not serve as mobile carrier of electrons in the murburn model, but serve as transient charge-storage agents (or capacitors) in and around the membrane (respectively).

Figure 4: Structure-function correlations of chloroplast/thylakoid membrane-embedded redox proteins, under ETC-CRAS and murburn purviews.

IV. Viability and verifiability of the murburn model of bioenergetics

While advocating the new theory, we have faced majorly two dismissive comments over the last four years. (1) “ROS is chaotic and will mess up life in an uncontrollable way. So, it is unlikely that such an uncontrolled reaction can give anything meaningful.” (2) “ROS is only in oxic systems. Since anoxic systems evolved before oxygen concentration became higher in earth’s atmosphere, the murburn model is irrelevant.” At the outset, it must be said that such comments stem from aesthetic disposition or inadequate awareness on the new theory. Most importantly, the first objection statement is invalid because the existence of ROS is a reality of cellular functioning and its presence cannot be wished away. If ROS were only toxic wastes, evolution need not have resorted to one-electron active agents that ensure ROS production. Safer two-

electron alternative modalities like substrate-level phosphorylations alone could have been used. But the observed realities and theoretical premises mandate the utilization of DRS in life processes. The murburn model is a simple chemical theory well-founded in thermodynamics, kinetics, probability, mechanistic, and evolutionary considerations. An example of selectivity of radical mediated oxidative reactions is when a cloth dipped in water+oil or a paper dipped in water+alcohol gets burnt. The fabric/paper gets charred only when the oil/alcohol supply runs out. Similarly, the collateral damage occurs over long periods in cellular systems that employ the murburn principles. Also, the fact that there are several ROS utilizing enzymes in the cells speaks of their utility, not of their danger (Jacob & Manoj, 2019)! A suitable analogy is- if we have gloves, cutting boards and knife-stands and knives in the kitchen, it does not mean that knives are merely lethal weapons of combat in kitchen. Also, quite contrary to perceptions, redox enzymes are not easily denatured by ROS and could be stable to them (Manoj & Hager, 2001). ROS mediate chaotic reactions only when present in large amounts at a given locus (at any instant), whereas they are very selective/specific in discretized generation and sustained release scenarios (Manoj, 2006; Manoj et al., 2020a). If a highly unstable ROS like nitric oxide can evolve to carry out the agenda of sensitive molecular messenger, why cannot other such species serve positive roles in routine physiological scenarios? The perception that '*ROS are dangerous!*' is a preconceived disposition, and lately this perception is changing, with more constructive functions emerging for the same (Mittler, 2017). Although, there is some collateral damage, the pros of the spontaneous and stochastic murburn scheme far outweigh the cons. Further, shift from anoxic to hypoxic or oxic environments (or vice versa) would be facile with the murburn bioenergetic principles, as the functionalism is dependent primarily on stochastic interactions/reactivity and is not contingent upon deterministic outcomes based on intermolecular recognition/affinity. While the formation of oxygen-centered radicals would be facile in oxic systems, there could be H/N/S centered reactive species in the anoxic systems. While the ETC-CRAS is a deterministic and essentially multi-molecular proposal, murburn model is based on stochastic/statistical principles and is bimolecular at the core. Thus, it can better explain the spontaneous origin from chaos, as the analogy it offers is akin to a hearth or spontaneous nuclear reactor (Manoj, 2018b). DRS mediated reactions can afford temporal selectivity/specificity and thus, sustenance amidst chaos (Manoj, 2006; Manoj et al., 2020a). Continuous evolution and trait differentiation through generations is aided by virtue of mutations and epigenesis induced

by DRS. Eventually, termination of life occurs due to the accumulation of unfavorable collateral/side reactions introduced by DRS, and thereby life ceases and disintegrates, returning to the larger domain of chaos. It is quite rational to consider that chaos can support a limited reign of order within, as seen manifested in fractal structures of several biological systems. If life can originate from/within chaos, it is logical to consider that chaos would have favorable elements for life-sustenance too! That is: all spontaneous reactions that occur in a cell may not be unfavorable, and murburn concept can be deemed as a compendium of the hitherto lesser considered (but favorable!) aspects of DRS mediated outcomes. It is evident (and also partly accepted now) that DRS also enabled trans-phase or organelle-organelle or cell-cell communications, thereby leading to multi-cellular assemblies and control systems.

Murburn model is the only standing mechanistic proposal that can explain cyanide's impact (dosage, kinetics, thermodynamics, etc.) in the porphyrin/flavin mediated physiologies within membrane systems, including aerobic respiration and oxygenic photosynthesis (Parashar et al., 2014, Manoj et al., 2016b-c; Manoj & Soman, 2020; Manoj et al., 2020a; Manoj & Bazhin, 2021). Since murburn model mandates a one-electron chemistry (for effective charge separation), the presence of several one-electron redox active agents on membrane-embedded proteins is justified. The murburn model successfully explains why Complexes I-III have long matrix-ward pendulous arms with redox-active centers, whereas the ETC-CRAS model cannot justify the evolutionary mandate for large apoprotein bulbs with multiple redox centers outside the trans-membrane region (Manoj et al., 2019a-b). Also, super/mega-complex or aggregate formation of redox proteins on endoplasmic reticulum or mitochondrial or chloroplast membranes and multimeric nature of the soluble hemoglobin are not explained within the classical perspective. Murburn concept explains these structural evolutions as stochastically selected events that enabled the proteins to better peruse the DRS generated in each other's vicinity (Manoj et al., 2019a, Parashar & Manoj, 2021, Parashar et al., 2021; Manoj et al., 2021b). A few simple and direct experimental agendas that ratify and delineate the new model for bioenergetic phosphorylations (particularly, within the lesser complicated system of oxidative phosphorylation or mitochondrial system) are listed:

1. Provision of NADH/oxygen would lead to an increase of DROS in the system. This observation would be an anti-thesis to the ETC-CRAS model's evolutionary relevance.

2. Increase of DROS within the system would correlate to increase in TMP/ATP synthesis. Such an observation would correlate TMP build-up as an outcome of ECS and DROS production and ATP synthesis within mitochondria; and not the other way around!
3. In situ/in vitro, provision of DROS could lead to the formation of ATP (from ADP/Pi). This observation would directly implicate DROS in ATP synthesis, while it would remain inexplicable how TMP can be tapped to give rise to an O-P bond in ATP.
4. As the model involves DROS, it is destined to be stochastic, non-integral and variable reaction outcomes are expected. This justifies the murburn equation of oxidative phosphorylation: $\text{NADH} + \text{H}^+ + \text{O}_2 + n\text{ADP} + n\text{Pi} \rightarrow \text{NAD}^+ + \text{H}_2\text{O}_2 + n\text{ATP} + n\text{H}_2\text{O}$. This equation shows that protons serve as a reactant in the oxidative phosphorylation reaction, thereby contributing to the overall energetics, a point completely missing in ETC-CRAS assumptions. Since the ETC-CRAS mechanism mandates deterministic ratios of electrons to protons and to ADP, the two mechanisms can be thus delineated.
5. Each one of the Complexes I-IV would have DROS-production abilities. Such an observation would be incompatible and inexplicable with ETC-CRAS model.
6. Each one of the Complexes I-IV would have ADP-binding sites. This is a necessary mandate of murburn model, whereas it is a superfluous aspect in ETC-CRAS.
7. Provision of ADP+Pi would increase NADH/O₂ consumption and DROS/ATP production. In the ETC-CRAS mechanism, since there is no direct coupling or thermodynamic pull concept, presence of ADP+Pi does not affect the ETC. Therefore, this is a simple way to delineate the two proposals.
8. ATP synthesis can be achieved or demonstrated in vesicles/mitochondria without pH gradient. This would demonstrate the physiological irrelevance of equilibrium-assisted synthesis via the equation: $\text{ADP} + \text{Pi} + \text{H}^+ \rightarrow \text{ATP} + \text{H}_2\text{O}$.
9. Inhibition of reaction setups 7 & 8 by catalytic amounts of cyanide can be noted. Since cyanide is a stoichiometric inhibitor in the classical scheme, this demonstration would ratify the murburn model. In the classical purview, if we add ADP+Pi to cyanide-blocked mitochondrial Complex IV, we should still see some ATP synthesis in the mitochondrial system because Complexes I & III can still pump protons.
10. Red blood corpuscles or erythrocytes form a major portion of human cellular counts. Lacking mitochondria and nucleus, they live about four months and produce mM levels of ATP.

Calculations show that glycolysis cannot explain such high concentrations of ATP and there is structural evidence to project that the outcome could owing be to hemoglobin serving as an ATP-synthesizing murzyme (Parashar et al., 2021).

V. Conclusions

Herein, holistic biological rationales are provided to show why the classical ETC-CRAS model's *pmf*/TMP-driven phosphorylation mechanism is untenable. Also presented are multitudes of living examples that lack the fundamental requisites sought by the ETC-CRAS paradigm. Further, using comparative analyses, the agendas ratifying the murburn proposals have also been delineated. Our recent works and re-interpretation of a vast amount of data available from literature disclaim the Kaelin-Mitchell-Boyer paradigm of ETC-CRAS. This development sets the stage for a serious indulgence of the alternative presented in murburn bioenergetics model. The murburn theory posits that redox proteins mediate ECS leading to DRS/ROS, which are the true catalytic agents driving ATP-synthesis at membrane interfaces in prokaryotes and within organelles (mitochondria/chloroplasts) of eukaryotes. DRS-based mechanisms allowed the origin of life, and they continue to sustain life and serve as the driving forces for the evolution and termination of life.

Declarations: The author has no conflict of interests to declare. The work was powered by Satyamjayatu: The Science & Ethics Foundation.

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