Review of: "The dual energetic supply of eukaryotic cells"

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"The dual energetic supply of eukaryotic cells". János Hunyady

According to the Abstract, in this manuscript the author "summarizes the defining elements of the structures responsible for energy transformation [SET]," specifically, the hypothetical SET-AG and SET-OP. Although not unambiguously defined, I believe that the author proposes that the SET-AG is responsible for anaerobic fermentation, whereas the SET-OP is responsible for aerobic oxidative phosphorylation. The author also discusses the role of HIF-1a (along with its oxygen-activated proteolysis) in anaerobic/aerobic metabolic switching, as well as in tissue regeneration, blood vessel growth (angiogenesis), apoptosis regulation, and the formation of pluripotent cells. In the Abstract, the author also mentions that "the same structures" increase the development and malignancy of cancer. It is not clear if the "structures" meant by the author here are HIF or SET. In any case, cancer development and malignancy are not in fact discussed anywhere in the manuscript.

Much of the chemistry and biochemistry described in this manuscript is either impossible (e.g., a reducing agent causing oxidation), unclear (e.g., no balanced chemical equations presented), and/or unsupported by literature. Given that the two SETs described by the author are the key focus of this manuscript, it is curious that the author doesn't describe what these structures are: Where are they located? What enzymes are contained in them? What proteins hold the structure together? Are they transient or permanent? And most importantly, what literature and experimental evidence support their existence?

For all of these reasons, I cannot support the publication of this manuscript. I include below critiques of specific passages and statements in the paper.

Abstract:

"*dual energy supply system*" What does this mean? Is it anaerobic fermentation vs. aerobic catabolism (e.g., glycolysis + citric acid cycle + oxidative phosphorulation)?

"*energy transformation*". Vague: transformation from what to what?

"*the way of renewal*" Vague. Not sure what the author means here.

Energy Transformation:

"It is well known that the energy supply of cells is provided by glycolysis (C6H12O6 = 6 CO2 + 6 H2O + 2880 kJ/mol). However, this is only partly true. In fact, ATP is also formed from sugar simultaneously when carbon dioxide is formed."

Again, the author's intent in these three sentences is unclear.

First, what does the author mean by "energy supply"? The production of ATP?

Second, **glycolysis** converts 1 glucose ($C_6H_{12}O_6$) to 2 pyruvate + 2 ATP + 2 NADH (not to 6 CO_2 + 6 H_2O). On the other hand, the equation for the **combustion** of glucose is:

$C_6H_{12}O_6$ **+ 6 O_2** 6 CO_2 + 6 H_2O $\Delta G°$ = -2880 kJ/mol

Lastly, ATP is not formed "from sugar", nor is it formed "simultaneously when carbon dioxide is formed." ATP is synthesized from ADP and phosphate via either

a. substrate level phosphorylation in glycolysis (2 ATP/glucose) and in the citric acid cycle (1 ATP/pyruvate = 2 ATP/glucose), which also produces CO_2; or by
b. oxidative phosphorylation: electron transfer through complexes I, III, IV, plus H+ flow through complex V (F1F0 ATP synthase)

"Energy transformation is realized in unique structures such as Structure for Energy Transformation (SET). Adenosine Diphosphate Producing Unit (ADP-PU) is the basic unit of SET [1]."

Again, it is unclear what the author means by energy "transformation." I have never heard of the author's hypothetical Structure for Energy Transformation (SET), nor of its basic unit, the Adenosine Diphosphate Producing Unit (ADP-PU). A Google search yielded only two papers, both written by this author, including his cited ref. 1. Based on the title of reference [1] given by the author in his Reference section, "*The Hypothesis of the Structures for Energy Transformation in Living Cells; Vitamin C, the Spark Plug of Glycolysis"*, I had hoped that this reference would describe the SET and ADP-

PU in some detail and present evidence supporting their existence. However, reference [1] contains no such detail nor any supporting evidence. In fact, the title of the published paper, "*The Result of Vitamin C Treatment of Patients with Cancer: Conditions Influencing the Effectiveness,"* is dramatically different from that cited in this manuscript.

*"the conditions for the creation of life"*Namely, what conditions?

"Among the many cells with different properties were blue algae, which continuously produced O2 molecules."

I think what the author meant to say was: "The earliest cells to produce oxygen were the cyanobacteria (a.k.a. blue-green algae), which evolved oxygen via photosynthesis."

"the cells were not prepared to protect against the highly destructive O2.

Which cells? Cyanobacteria would have evolved resistance to oxygen damage. Presumably the author means ancient fermenting microorganisms.

On p. 2 the author wrote *"The advantages of symbiosis are significantly more energy, protection against free radicals, and regeneration ability of organisms [3]."*

And then just a few lines down, *"Mitochondria provide the cell with protection against free radicals and a significantly better energy supply...[2].*"

This is redundant.

"Due to the lack of O2 caused by injury or any cause, the hydrolysis of the Hypoxia-Induced Factor (HIF)-1α is cancelled.

How does this work? Is there an**oxygen-activated HIF protease**? "Cancelled" is a poor word choice; "inhibited" is better.

"the HIF system influences about 200 genes, converting cells to ancient energetics."

What references support this statement? What does the author mean by "ancient energetics"? Anaerobic fermentation?

"circulation will be restored with the help of newly-formed blood vessels."

Is angiogenesis triggered by HIF-activated genes?

"SET-AG (belonging to the ancestral cell) and SET-OP (belonging to the mitochondria). The operational activity of these structures can be determined"

What does “AG” stand for? Does “OP” stand for oxphos? Are these SETs specific structures? By “SET-AG”, doesn’t the author simply mean the enzymes that carry out anaerobic fermentation (e.g., lactate dehydrogenase or pyruvate decarboxylase), and SET-OP are the enzymes that carry out oxidative phosphorylation (e.g., pyruvate dehydrogenase and the electron transfer complexes)? If so, I am unaware of any evidence that these are specific structures; rather, they are collections of enzymes. Having said that, there is some evidence transient super-complexes comprising two or three enzymes can form, e.g., glycolysis enzymes, and mitochondrial inner membrane electron transfer complexes.

“In an oxygenated environment, SET-AG will not function, as the ATP produced by the mitochondria significantly exceeds the capacity of SET-AG, resulting in the shutdown of its activity.”

The author seems to state here, without any supporting citations, that high [ATP] inhibits fermentation enzymes. Is this true? If so, what references support this?

On the bottom of p. 2 the author wrote *“Due to the lack of O2 caused by injury or any cause, the hydrolysis of the Hypoxia-Induced Factor (HIF)-1α is cancelled. Subsequently, the HIF system influences about 200 genes, converting cells to ancient energetics.”*

And then just a few lines later *“In an anoxic or hypoxic environment, mitochondria stop working. At the same time, there is no hydrolysis of HIF-1 alpha, which will result in the activation of the SET-AG.”*

This is redundant.

Finally, how exactly does HIF activate fermentation enzymes and inactivate oxidative phosphorylation enzymes? My impression has always been that this switch from aerobic to anaerobic is controlled simply by the cytoplasmic concentration ratio of [NAD+]/[NADH]. The common reasoning is that NADH is a substrate for the reduction of pyruvate to lactate by lactate dehydrogenase, whereas the oxidant NAD+ is a substrate for all citric acid cycle enzymes including pyruvate dehydrogenase. In the absence of O2 as the terminal electron acceptor of the mitochondrial inner membrane electron transfer complexes, NAD builds up as NADH. The lack of NAD+ substrate inhibits all of the citric acid cycle enzymes, causing pyruvate to build up. This in turn, along with the excess of NADH substrate, activates lactate dehydrogenase and thus fermentation.

“In the case of anoxia, ADP-PU produces ten CO2, while in an oxygenated environment, SET-OP forms 9 X (ten + six CO2). Accordingly, the energy production capacity of SET-AG is significantly lower than that of SET-OP.”

The author gives us no explanation as to what reaction(s) is/are carried out by the “ADP-PU”, hence the reader has no

idea where these “ten CO2” come from.

Nor, for that matter, is it clear how the *“SET-OP forms 9 X (ten + six CO2).”*

Finally, “energy production capacity” is measured by ATP synthesis, not by CO2 release.

The author wrote: *“P-cluster of nitrogenase (8Fe-7+6S))”* but what does “8Fe-7+6S” mean? Presumably, from Fig. 2, the author meant to write: $Fe_8S_7(cys\text{-}S)_6$. Also, I have never heard of an Fe_6S_6 cluster. According to Wikipedia, for example, “Fe–S clusters can be classified according to their Fe:S stoichiometry [2Fe–2S], [4Fe–3S], [3Fe–4S], and [4Fe–4S].[6]”

The information in Table 1 is found in any General Chemistry textbook. It seems unnecessary here. Having said that, the author’s Fe listing is wrong. According to the author’s TABLE 1, Fe can have a total of either 26, 27, or 28 electrons, but with 26 protons, that would mean that iron could be either 0, -1, or -2. That is clearly wrong.

The electron configuration of elemental Fe is $[Ar]4s^23d^6$; Fe^{2+} is $[Ar]3d^6$; and Fe^{3+} is $[Ar]3d^5$. Hence, iron’s fourth shell either contains 2 electrons (in elemental iron), or no electrons (in oxidized iron); iron’s third shell either contains 13 electrons (Fe^{3+}) or 14 electrons. Hence, the author’s statement *“The outermost electron shell of iron can contain two, three, or four electrons (Table 1).” i*s incorrect. Fe_xS_x clusters always contain oxidized iron cations, which never have any electrons in their outer (4^{th}) shell.

“The binding affinity of iron to oxygen and sulphur is determined by the number of electrons in the outer electron shell of iron. In the case of two electrons, the iron binds the oxygen, while in the case of three, the sulphur bind [sic] *is realized. The process produces four Oe2-, producing two CO2 or four H2O molecules.”*

”

I don’t understand exactly what the author is saying here, but I believe that in the first two sentences he opines that Fe^{2+} (the author’s “3” electrons in the outer shell are actually $3d^64s^0$) binds oxygen (only??) while Fe^{3+} (the author’s “2” electrons in the outer shell are actually $3d^54s^0$) binds sulfur (only??). There are several things wrong with this. First of all, iron cations spontaneously bind either oxygen or sulfur, hence to state that one form of iron binds “the oxygen, while” the other form “the sulfur” is inaccurate. It’s not an either/or situation, but rather a matter of degree, i.e., which is more spontaneous.

Secondly, the author gives no citations here. How do we know that this is true?

Thirdly, this statement is quite unlikely to be true. Iron cations bind hydroxide tightly $K_{sp} \approx 10^{-15}$ for $Fe(OH)_2$, 10^{-36} for $Fe(OH)_3$, and they bind to oxide (O^{2-}) so strongly that K_{sp} for FeO and Fe_2O_3 cannot be measured. On the other hand, iron cations bind only weakly to hydrosulfide (HS^-); instead, iron cations react with HS^- to yield the sulfide precipitate plus H^+: $K_{sp} = 3.7(10^{-19})$ for FeS, $1.4(10^{-88})$ for Fe_2S_3. The fact that iron hydroxides are insoluble while iron hydrosulfides are soluble, while iron sulfides have measurable K_{sp} values whereas iron oxides do not, suggests that both iron(II) and iron(III) bind oxide (and hydroxide) more strongly that they bind sulfide (and hydrosulfide). This also makes sense because oxide is a much smaller anion than sulfide, hence it would form a stronger ionic bond with either iron cation.

And finally, I have no idea what the 'e' in "O^{e2-}" means. Does the author simply mean the oxide anion, O^{2-}? If not, then what? If so, then why add the 'e'? Furthermore, the author does not specify what "process" produces "two CO2 or four H2O molecules." This is mystifying. What is the reactant? Give a balanced chemical equation so the reader knows what you're proposing.

"The binding change mechanism of Complex V involves the active site of a β subunit's cycling between threestates. In the "open" state, ADP and phosphate enter Complex V; in Illustration 8, this is shown in white. The enzyme then changes shape and forces these molecules together, with the active site in the resulting "tight" state (shown in yellow) binding the newly produced ATP molecule. Finally, the active site cycles to the loose state (red) and will be ready for the next cycle of ATP production"

I could not find Illustration 8. Also, it is unclear why this information is included here. What does Complex V have to do with the arguments being made?

" the change of AA to DHA regulates the modulation of the electron shell of Fe.[11,12]

Is AA ascorbic acid and DHA dehydroascorbic acid? I read refs. 11 and 12 and could not find in them any statements relating to AA or DHA modulating "*the electron shell of Fe".*

The author needs to clarify what he means in this statement.

"The way of action, assumed by me: the two OH on the lactone ring of vitamin C are exchanged with the two central sulphur atoms of the 2Fe-2S sulphur-iron cluster, forming two Fe2+ from the two Fe3+, while it turns into Dehydro Ascorbate (DHA), and two H+ are formed. As a result of the change in the properties of iron, the four other sulphur atoms are replaced by molecules containing oxygen atoms. Oxygen atoms of two uric acids (UAs) (or two NH2-UAs) and two

H2PO4e- will occupy the place of the four sulphur atoms. After that, two hydrogen atoms of the two H2PO4e- molecules transform DHA into AA, while the iron atoms become three-electronic again (Illustration 3). This change results in a sulphur-oxygen exchange, creating four Oe2-, which leads to CO2 or H2O molecules forming. A similar reaction might run on regarding the two OH of the ribose part of the ATP when initiating one Fe-S cluster."

Illustration 3 is confusing and seems wrong. Given that Szen = carbon; Ken = sulfur; Vas = iron, then it looks like the author has included four ethyl thiol ligands instead of four cysteines in the Fe2S2 cluster depicted on the left (Fe2S2 + AA).

It seems like the next intermediate after the left-structure should actually be the right-most structure, which is incorrectly labeled "DHA: dehydro C vitamin". Here, an H on each of the four Et-SH groups has been replaced by an OH, but how??? What is the source of the OH? This is an extremely difficult oxidation reaction (CH COH) to catalyze.

The author wrote: *"the four other sulphur atoms are replaced by molecules containing oxygen atoms. Oxygen atoms of two uric acids (UAs) (or two NH2-UAs) and two H2PO4e- will occupy the place of the four sulphur atoms."*

The left-most structure does not depict this; there are no uric acids nor phosphates in the structure. Instead there are four "O^{e2-}" groups attached to a carbon; as depicted, these are actually C-OH hydroxyl groups.

The final structure in this sequence would then be the middle (mislabeled AA-C vitamin), in which there are two problems:

a. the bond between the two carbons in the lactone ring of the DHA is missing,
b. The Fe^{2+}-SEt bond has been broken, replaced by an -OEt(SH) bond; although this would be a spontaneous reaction, it is likely to have a very high activation energy and thus would be very slow.

The author seems to be calling for "vitamin C", i.e., reduced ascorbic acid, to bind in place of the two central sulfide anions in the Fe2S2 cluster, and oxidize the two Fe^{2+} to two Fe^{3+}. Even if reduced ascorbate could do this (it can't: ascorbate is a reducing agent, not an oxidizing agent), the Fe2S2 cluster can only lose a single electron. This is quite well-established.

Furthermore, the author proposes that *"two hydrogen atoms of the two H2PO4e- molecules transform DHA into AA"* But the hydrogens on $H_2PO_4^-$ are protons (H^+); in order to oxidize DHA to AA, they would have to be reduced to H· atoms or to hydride anion ($H:^-$). There is no known reaction where phosphate protons can do this.

Finally, as mentioned above, the meaning of the term "O^{e2-}" is obscure, hence how this could "lead to CO2 or H2O molecules forming" is also mysterious and unexplained.

Regarding the key section "**Structure of SET-AG and SET-OP**," I was unable to follow any of the chemical transformations delineated. They seem chemically impossible to me. In any case, one can't propose complicated reactions like these without giving balanced chemical equations and without citing supporting literature.

"The HIF system is the detector and conductor of the oxygenated and oxygen-free environment. It facilitates the cell back to ancient times. The HIF system ensures adaptation to an environment without O2"

What does the author mean by "conductor"? How can a cell be "facilitated… back to ancient times"?

"In the existence [sic] *of O2, the SET of Oxidative Phosphorylation (SET-OP) presents*[sic] *oxidative phosphorylation."*

I believe the author means "In the presence of O2, the SET of Oxidative Phosphorylation (SET-OP) catalyzes oxidative phosphorylation."

"cells use aerobic glycolysis offered by the Structure for Energy Transformation (SET)-Aerobic Glycolysis (SET-AG) in an anoxic environment"

There is no such thing as "aerobic glycolysis". Glycolysis converts glucose to pyruvate under both aerobic and anaerobic conditions. The difference is that under anaerobic conditions the pyruvate is reduced (e.g., to lactate or ethanol), while under aerobic conditions pyruvate is oxidized to acetyl-CoA and then further oxidized to CO2.

The section "Ribose" seems irrelevant and unrelated to anything else discussed in this manuscript.

I do not understand any of the four entries in the table in the final section, *"The efficiency of SET-AG and SET-OP".*

If the author is claiming in the final row that complete oxidation of a single glucose via oxphos yields 36 ATP, that is incorrect. The actual value in mammals is either 34.5 or 30.5 ATP/glucose, depending on whether glycolytic NADH is imported into the mitochondrial as NADH or FADH2, respectively. {See, for example, *J Bioenerg Biomembr* (2014)

46:229–241; DOI 10.1007/s10863-014-9547-y}