Research Article

The Energetic Systems of Eukaryotic Cells

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Background: We have recently published a new hypothesis proposing an alternative mechanism for the synthesis of ATP by involving specific cellular structures: Structure for Energy Transformation (SET).

Hypothesis: The SET, consisting of a multiplex electron transfer chain, potentially facilitates a chemical process involving Fe-S clusters, D-glucose, uric acid (UA), NO, and $H_2PO_4^-$ molecules. This leads to energy production and ATP, CO_2, and H^+ synthesis. The SETs of aerobic glycolysis (SET-AGs) are located in the peroxisomes, whereas the SETs of oxidative phosphorylation (SET-OPs) are located in the mitochondria of the eukaryotes. While this hypothesis suggests a potential alternative pathway for ATP synthesis, further experimental validation is required to confirm its mechanisms and physiological relevance.

Objective: To outline and explore the new hypothesis that ATP synthesis occurs through a complex process within the SET, involving multiple chemical constituents in a distinct stoichiometry, producing ATP, PO_3^{3-}, (Pi), CO_2, H^+, and energy.

Conclusion: Using adenosine diphosphate (ADP) and inorganic phosphate (Pi), ATP synthase catalyses the formation of adenosine triphosphate (ATP). The hypothesis explains the origin of ADP, Pi, and the energy required for ATP production. It also suggests a mechanism of action for cancer treatment with intravenous high-dose vitamin C therapy.

Energy transformation

Oil cannot be used directly by cars. It has to be transformed into petrol and diesel fluid. In the same way, cells convert glucose into ATP.

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Known data

Peroxisome, Mitochondrion, Structure for Energy Transformation

In primitive cells, energy may have been produced in the cell membrane. Later, a structure specialized in this area. The mitochondrion is an organelle found in the cells of most eukaryotes, including animals, plants and fungi. Lynn Margulis (1938-2011) proposed that the ancestor of eukaryotic cells avoided being destroyed by oxygen by entering into a symbiotic relationship with an aerobic bacterium. This gave rise to the mitochondria of eukaryotes[1][2]. Thus, eukaryotes have genetic material from both cells and can live in hypoxic and normoxic environments using the SET-AG energy conversion structure for aerobic glycolysis of the ancestral cell and the SET-OP energy conversion structure for oxidative phosphorylation of the mitochondrion. All eukaryotic cells contain mitochondria.

Properties of the atoms of the protonated adenine molecule

Turecek and Chen studied the features of the atoms of the protonated adenine molecule in the gas phase, water clusters, and bulk aqueous solution. They calculated that proton in the adenine ion 1^+ undergoes rapid migrations between positions N1, C2, N3, N10, N7, C8, and N9, resulting in an exchange of hydrogen atoms before the loss of a hydrogen atom forming an adenine cation radical at a dissociation threshold energy of 415 kJ mol^{-1}[3]. The proton displacement signals the path of the electrons in the adenine molecule. Thus, according to the results of Turecek and Chen, the adenine molecule has two entry points for electrons (N1 and N7), while electrons can leave the molecule through the N10 atom[3]. We assume that in the NH_2-UA molecules, C2 and C8 are the entry points for the electrons, and N10 is the exit point for the electrons.

Mitochondria contain vitamin C

Korth et al. suggested that two L-vitamin C molecules are located in the NADPH pocket, presumably near the adenine binding site in the inner membrane of the mitochondria[4]. Their conclusion is based on molecular mechanistic docking computations.

Electron transport chain

An electron transport chain (ETC) is a series of membrane-bound protein complexes (Complex I, Complex II, Complex III and Complex IV) and other molecules that transfer electrons from electron

donors to electron acceptors via redox reactions (both reduction and oxidation occur simultaneously) and couple this electron transfer with the transfer of protons (H^+ ions) across a membrane. Four [Fe_2S_2], one [Fe_3S_4], and seven [Fe_4S_4] clusters provided the proper function of Complex I, Complex II, and Complex III, as described by Austin et al.[5].

Fe-S clusters

Many Fe-S clusters are well known in organometallic chemistry as precursors to synthetic analogues of the biological clusters. In the simplest polymetallic system, the [Fe_2S_2] cluster consists of two iron ions bridged by two sulfide ions and coordinated by four cysteine ligands (in Fe_2S_2 ferredoxins).

The most common Fe-S clusters are of the rhombic [Fe_2-S_2] and cubic [Fe_4-S_4] types, but [Fe_3–S_4] [Fe_4–S_3] and [Fe_8-S_7] clusters of nitrogenase have also been described.

Cysteine

Cysteine (Cys) is a semi-essential proteinogenic amino acid with the formula HOOC-CH(-NH_2)-CH_2-SH ($C_3H_7NO_2S$) (Figure 1). Cys is a deterministic part of all Fe-S clusters, as it provides the continuity of the electron transfer in the Multiplex Electron Transfer Chain (METC). We assume that the Fe-S clusters form a continuous electron chain by connecting through their Cys parts. Two cys of two Fe-S clusters can create continuity between the clusters, as shown in Figure 1. The Nitrogen-Oxygen connection results in an N-C bond + H_2O, leaving one C-NH_2 and one C=O.

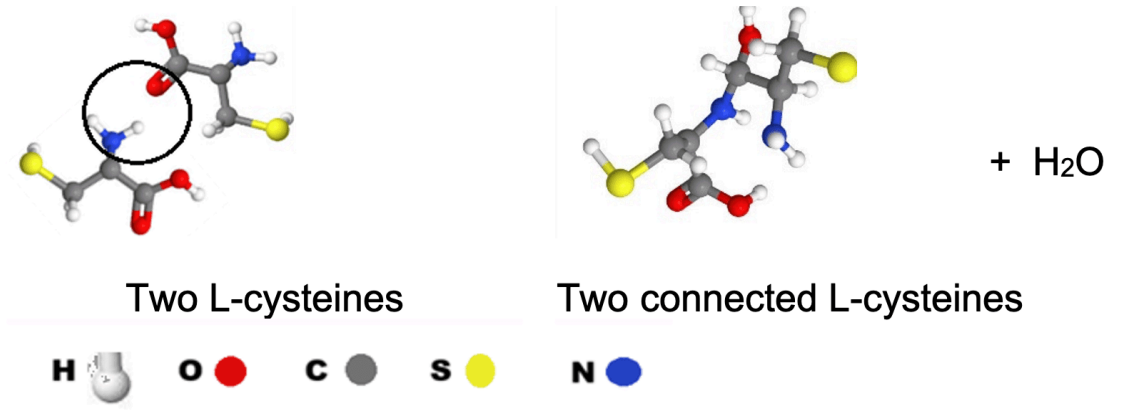

Figure 1. Connection of two L-cysteines

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The oxygen, sulfur and iron properties allow the oxygen and sulfur atoms to exchange places in the Fe-S cluster. The exchange is mediated by the flow of electrons facilitated by the nearby molecules.

Fe-S clusters, the Fe^{3+} - Fe^{2+} change

Fe-S clusters are the essential structures in the electron transfer chain. The central part of all Fe-S molecules is [Fe_2-S_2]. The Fe atom can have 23 (Fe^{3+}) or 24 electrons (Fe^{2+}). Each [Fe-S] cluster has two Fe atoms bound to two sulfur atoms, and each Fe atom is bound to two cys-S components (HOOC-CH(-NH_2)-CH_2-S-R)[6]. These two Fe atoms form the activation point of the Fe-S clusters.

ATP synthesis

According to present knowledge, ATP is produced by rotator catalysis in the ATP synthase, which is an ancient nanomachine. The mitochondrial F1 and FO regions of ATP synthase are the most prominent protein complexes in the mitochondrial cristae. ATP synthase. It uses the electrochemical proton gradient across the inner mitochondrial membrane to produce ATP by rotator catalysis[7][8][9].

ATP Synthesis in the Structures for Energy Transformation

Determining Constituents of Structure for Energy Transformation

Nitrogen monoxide. Amination of uric acid

Nitrogen monoxide (NO) is an important neurotransmitter in the mammalian body. It can also be found in the cells of bacteria, plants, fungi and animals, and it plays an important regulatory role in processes such as ATP production and cellular respiration, apoptosis, photosynthesis, phagocytosis, and cell movement.

Recent advances in NO biology show that NO is generated in different cell compartments, including specific plasma membrane regions, mitochondria, chloroplasts, peroxisomes, the Golgi complex and intracellular membrane systems[10].

We assume that $[Fe_8S_7(C_3H_7NO_2S)_6]^{2-}$ clusters of nitrogenase take up oxygen atoms from the C1 position of UA molecules. At the same time, nitrogen is provided by the same $[Fe_8S_7(C_3H_7NO_2S)_6]^{2-}$ cluster of nitrogenase. Thus, UA is nitrified with the help of the aminotransferase enzyme, producing an aminated UA and 4 CO_2 molecules.

$4\ UA + 4\ NO + 4\ H_2PO_4^- + 2$ acetic acids $= 4\ NH_2\text{-}UA + 4\ CO_2 + 4\ PO_3^{3-}$

The reaction is mediated by aminotransferase and four $[Fe_8S_7\ (C_3H_7NO_2S)_6]^{2-}$ clusters.

Energy transformation

Evolution of the Energetic system in the living world

The maintenance of life requires a continuous flow of electrons. Eukaryotic cells provide this through continuous glycolysis. In cells, two glucose molecules are converted into one molecule of adenosine triphosphate (ATP), two molecules of CO_2, one molecule of acetic acid, and one molecule of Pyruvate and energy.

Vitamin C and ATP are the activators and initiators of energy transformation

Sulfur – Oxygen change

Kinga Linowiecka et al. found that L-ascorbic acid (AA) is a sensor of oxidative stress and a regulator of gene expression. They also pointed out that the conversion of AA to dehydro-ascorbic acid (DHA) regulates the modulation of the electron state of iron in Fe^{2+}-dependent dioxygenases[11].

Two electrons enter the Fe-S cluster, resulting in a Fe^{3+} - Fe^{2+} modification. As a result, the OH of AA or ATP will replace the S of the Fe-S cluster, and two H^+ will leave the cluster, creating the membrane potential[11]. Later in the Fe-S cluster, Fe^{2+} will become Fe^{3+} by hydrogen atoms from $H_2PO_4^-$.

The base of the continuous electron transfer

$Fe_2\text{-}S_2$ + four H_2PO_4 + acetic acid $= 4\ X\ [2H^+ + PO_3^{3-} + 2\ e^-] + 2\ CO_2 + H_2O$

$2\ Fe^{3+} + 2\ e^- = 2\ Fe^{2+}$

The two OHs on the AA and ribose of an ATP molecule can change the S to OH in the Fe-S clusters. First, ATP and AA activate the Fe-S clusters by two OH, resulting in electron transfer. The cluster is then ready to function.

The sulfur–oxygen exchange caused by AA is demonstrated in Illustration 2.

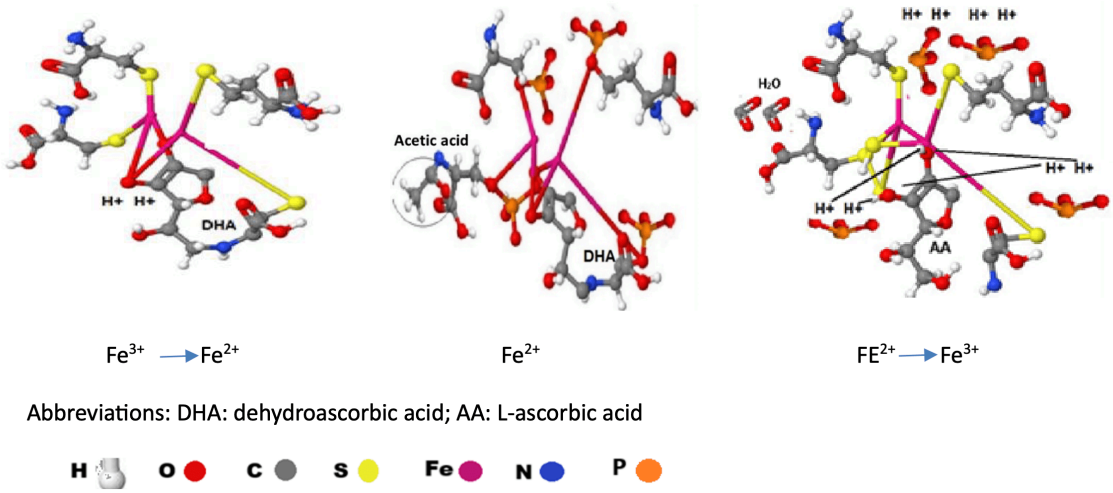

Figure 2. In Fe-S clusters, ascorbic acid leads to a sulfur-oxygen change.

ATP-uric acid-ATP cycle

The view that UA is the end-product of ATP metabolism is widely accepted. Gounaris et al. described that the hypoxanthine molecule could evolve as an effective capture of inorganic nitrogen species. These authors have also reported that hypoxanthine, the biochemical precursor of adenine and guanine, trapped nitrite ions and was reductively converted to adenine[12]. A similar reaction may occur with UA amination. We predict that UA is not an end-product but one element of an ATP – UA – NH_2UA – adenosine – ADP – ATP - UA cycle. Proof of the ATP-uric acid-ATP cycle concept using radiolabeled UA is in progress[13].

The hypothesis of the structure for energy transformation

We have created a hypothetical structure responsible for the production of ATP[14]. The hypothesis is based on known data such as the oxidative pentose pathway, the electron transport system in mitochondria[15], the biochemical nature of $H_2PO_4^-$, the results of Turecek and Chen on the nature of adenine[3], and the oxidative experimental results supported by stoichiometric calculations suggesting that there are two vitamin C molecules in the NADPH pocket of the mitochondrion[4]. The JSME Editor, courtesy of Peter Ertl and Bruno Bienfait, helped to calculate the 3D structures of the hypothetical vision of SET[16].

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The first hypothesis has been further developed[17][18][19][20][21][22]. This paper presents the final hypothesis.

Our hypothetical SET consists of a modified version of the well-known complexes I, II, III, IV and V. SET is the engine of the five multienzyme complexes in the peroxisomes and the mitochondria. Thus, the concept of SET helps to understand the energetics of eukaryotic cells.

We believe that SETs provide the cell with new ATP molecules and afford energy supply and H^+ in eukaryotes. Units of SETs are made up of the same building blocks.

The constituent elements of the structures of energy transformation

ATP synthase

ATP synthase forms rows of dimers in the cristae membranes[7][8][9]. Carrier molecules carry ADP into the nanomachine, where, with the help of chemical energy in food, phosphate can be added to produce ATP[23]. The origin of ADP, Pi and energy is not explained in detail, while the present hypothesis answers these questions.

Pyruvate dehydrogenase

The Pyruvate dehydrogenase complex is a three-enzyme complex that converts pyruvate to acetyl-CoA by decarboxylating pyruvate. Acetyl-CoA can then be used in the citric acid cycle to fuel cellular respiration, and this complex links the glycolysis metabolic pathway to the citric acid cycle. Pyruvate decarboxylation is also known as the "pyruvate dehydrogenase reaction" because it involves the oxidation of pyruvate[24].

Building molecules of the electron transfer chain

The energy supply structures contain the mitochondrial multienzyme complexes. They are made up of permanent components that form a nest waiting for the source molecules. Fe-S clusters, Flavine, and nicotinamide are the main components of the nest. This structure organizes the mitochondrial multienzyme complexes into functional units.

The supposed structures for energy transformation

Electron Transfer Chain

Austin et al. described that four [Fe_2-S_2], one [Fe_3-S_4], and seven [Fe_4-S_4] clusters provide the proper function of Complex I, Complex II, and Complex III[5]. We assume that instead of the seven [Fe_4-S_4($(C_3H_7NO_2S)_4$]$^{2-}$ clusters, one [Fe_3-$S_4(C_3H_7NO_2S)_4$]$^{2-}$, one [Fe_4-$S_4(C_3H_7NO_2S)_4$]$^{2-}$ + four [Fe_8S_7 $(C_3H_7NO_2S)_6$]$^{2-}$ and four [Fe_2-$S_2(C_3H_7NO_2S)_4$]$^{2-}$ clusters form the electron transfer chain. We calculate with [Fe_8S_7] because it helps in the amination of the uric acids.

We propose that the four [Fe_8S_7 $(C_3H_7NO_2S)_6$]$^{2-}$ clusters of nitrogenase and four [Fe_2-$S_2(C_3H_7NO_2S)_4$]$^{2-}$ clusters form a ring connected to the [Fe_3-S_4] and [Fe_4-S_4] clusters. The Fe_3S_4 cluster bounds two [Fe_8S_7] clusters of one ETC, while the Fe_4S_4 cluster bounds the other two [Fe_8S_7] clusters of the neighboring ETC. The interconnection of the ten Fe-S clusters forms the **electron transfer chain (ETC)** (Figure 4).

The four [Fe_2-S_2] clusters and the [Fe_3-S_4] cluster have one (4+1) activation point, the [Fe4-S4] cluster has two activation points, and the four [Fe_8-S_7] cluster has four (4x4) activation points (5+2+16=23).

Twenty molecules of ATP and three molecules of AA activate the ETC reaction. Two OH of the ribose on the ATP will bond to two Fe atoms of the [$Fe_8S_7(C_3H_7NO_2S)_6$]$^{2-}$, while the NH_2 part of the adenine of the ATP's adenine will bound to the C=O part of the linker. In this way, the 20 molecules of ATP bond to the 20 C=Os of the bound cysteines.

In the [Fe_8-S_7] 1, [Fe_8-S_7] 3, and [Fe_4-S_4] clusters, three AAs are responsible for activation. The AA connects the C-NH_2 part of the cys. (Figure 4).

Multiplex electron transfer chain

The [Fe_3-S_4], [Fe_4-S_4], [Fe_8-S_7] 1, and [Fe_8-S_7] 3 clusters bind the neighboring ETCs. They form a **multiplexed electron transfer chain with two ETCs** (METC) (Figures 4 and 5)

Structure of Energy Transformation for Aerobic Glycolysis

The ATP synthase enzymes and two ETCs form the Structure of Energy Transformation for Aerobic Glycolysis (SET-AG).

Structure of Energy Transformation for Oxidative Phosphorylation

The Structure of Energy Transformation for Oxidative Phosphorylation (SET-OP) is formed by SET-AG + pyruvate dehydrogenase complex.

The SET-AG is in the peroxisomes, while SET-OP is in the mitochondria.

Activation and initiation of the multiplex electron transfer chain.

The Fe-S clusters need to be activated and initiated by ATP and AA to continue the constant electron transfer.

The $[Fe_8S_7(C_3H_7NO_2S)_6]^{2-}$ clusters have six cys parts. It thus offers places for six oxygen-containing molecules. The cluster bounds one UA by the C6-positioned oxygen, two aminated UA by the C2 or C8-positioned oxygen, one NO molecule and two $H_2PO_4^-$ molecules by the double-bonded oxygen of the molecules.

Four aminated uric acid molecules linked to nicotinamide and Flavine molecules link the four $[Fe_8S_7(C_3H_7NO_2S)_6]^{2-}$ clusters of nitrogenase (Figure 3). The four NO, four UA, eight D-glucose four F_2S_2, the F_3S_4, the Fe_4S_4 clusters and the 32 $H_2PO_4^-$ molecules are not shown.

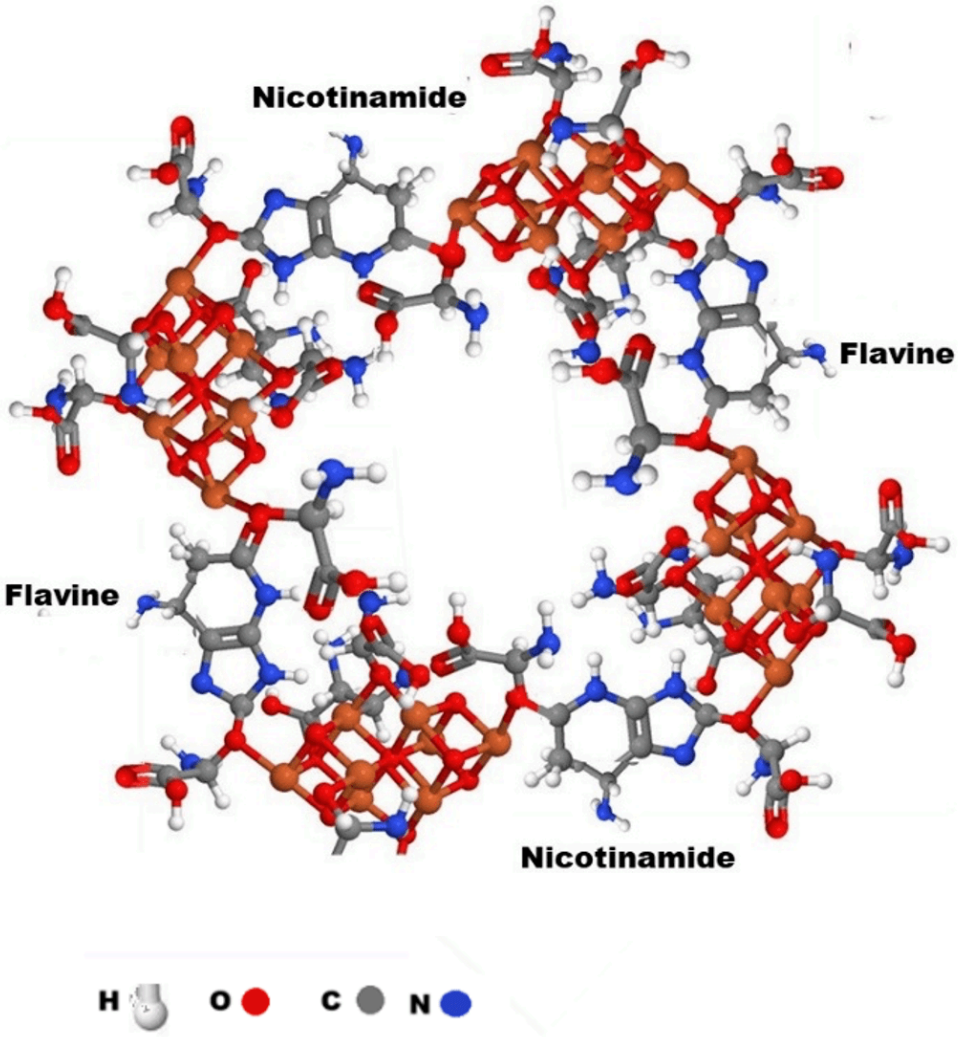

Figure 3. Four aminated uric acid molecules connected to nicotinamide and Flavine molecules link the four $[Fe_8S_7\,(C_3H_7NO_2S)_6]^{2-}$ clusters of nitrogenase.

We assume that the SET-AG forms four new ATP molecules. The amination of UA, the ribose bond to the UA-originated adenine, and the three Pi bounds all require energy, which is provided by five ATPs and carbon oxidation.

The energy transformation in one ETC results in four new ATP molecules. The formation of four ATP requires the energy of 4X5 ATP + energy of 24 or 36 carbon oxidations. So, 20 ATP must go into the ETC. 2 X 4 ATP activates the second and fourth $[Fe_8S_7]$, 2 X 3 ATP is used to activate the first and third $[Fe_8S_7]$, and 6 ATP are needed to activate the four $[Fe_2S_2]$, the $[Fe_3S_4]$ and the $[Fe_4S_4]$ clusters. Three AAs bound to the first, third $[Fe_8S_7]$ and the $[Fe_4S_4]$ clusters initiate the electron transfer (Figure 4).

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Binding points of the multiplex electron transfer chain.

An METC contains two ETCs. Neighboring ETCs are connected by the [Fe_3-S_4], [Fe_4-S_4]' [Fe_8-S_7] 1, and [Fe_8-S_7] 3, clusters (Figure 4 and 5).

Source molecules of the SETs

Activation of the ETC transforms sulfur atoms into oxygen-containing molecules, such as H2PO4-, NO, UA, and NH2-UA. In addition, D-glucose and acetic acid molecules will be bound to the connecting cys part of the Fe-S clusters (Figure 4). From the source molecules (D-glucose, UA, NO, $H_2PO_4^-$), new ATP molecules and membrane potential (H^+), acetic acid, and Pyruvate are formed, and CO_2 + energy is released.

The ETC of the SET-AG is shown in Figure 4. The eight D-glucose molecules are not shown. The chain consists of twenty-four connecting points. Sixteen links are between the four $[Fe_8S_7\ (C_3H_7NO_2S)_6]^{2-}$ and four $[Fe_2\text{-}S_2(C_3H_7NO_2S)_4]^{2-}$ clusters (⋄1-16); two Links (□1,2) are between two [Fe_8-S_7] and [Fe_4-S_4] clusters, and another two (△1,2) are between the [Fe_3-S_4] and two [Fe_8S_7] clusters. The remaining 4 cys connect the neighbor ETC clusters through the [Fe_3S_4] and [Fe_4S_4] clusters (⬡ME1, ME2, ME3, ME4) to form the METC. The M5, M6, M7 and M8 connect ETC1 to the ETC2. (Figures 4 and 5).

The 24 bonds provide 24 NH_2 and 24 C=O sites. Twenty of the C=O sites are bound by the NH_2 of the adenosine in the ATP. The NH_2 bounds eight D-glucose, eight acetic acid, two Flavine, and three AA molecules. Two AAs activate the F_8-S_7 1 and the F_8-S_7 3 clusters, while another AA, bound to the ME3 and ME7 linkages, activate the two [Fe_4S_4] clusters (Figures 4 and 5).

Oxygen binding points of multiplex electron transfer chain.

Oxygen-containing molecules will replace the sulfur atoms after the ETC is initiated. The ETC has 48 cys structures. The four [Fe_8S_7] clusters contain 6x4=24, the four [Fe_2-S_2], the [Fe_3-S_4], and the [Fe_4-S_4] clusters contain 6x4=24 cys, providing 48 oxygen binding points (Table I).

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Structure molecules	Source molecules				O binding point
	UA	NH_2-UA	$H_2PO_4^-$	NO	
4 Fe_8-S_7	4	4 x 2	8	4	6x4 =24
4 Fe_2-S_2			16		4x4=16
1 Fe_4-S_4			4		1x4=4
1 Fe_3-S_4			4		1X4=4
ETC	4	4x2=8	32	4	48
2 Flavine					
2 Nicotinamide					

Table I The electron transfer chain's structure, source molecules and oxygen-bounded points

Abbreviation: UA: uric acid; NH_2-UA: nitrified UA; ETC: electron transfer chain.

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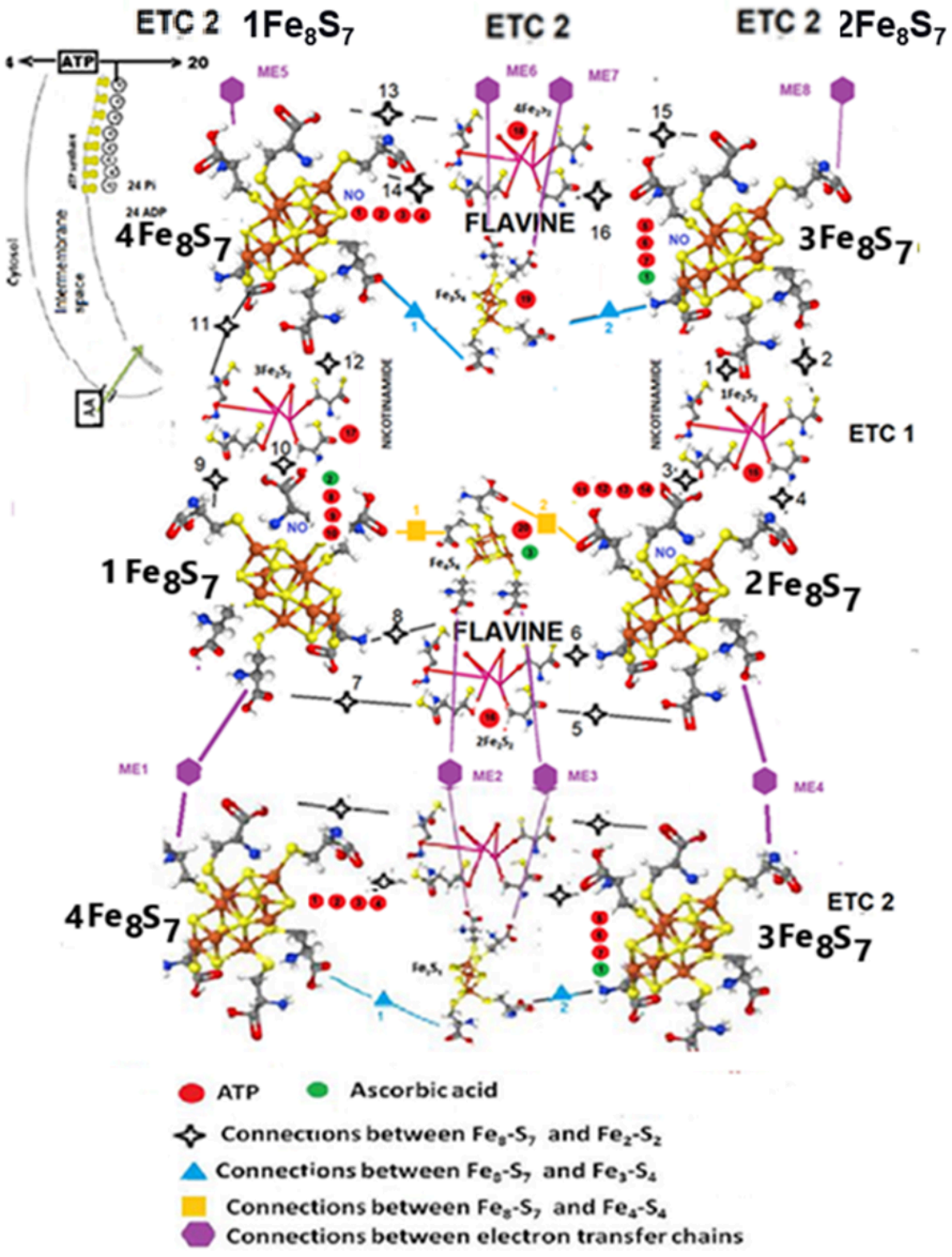

Figure 4. The electron transfer chain of the SET-AG, the eight D-glucose and thirty-two H_2PO_4 molecules are not shown.

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Results of the energy-producing units

Table II summarizes the relationship of the oxygen binding points to the oxygen-containing molecules and the results of ETC. The molecules involved are 4 UA, 4 NH_2-UA, 32 $H_2PO_4^-$, and 4 NO + 8 D-glucose.

Fe-S clusters	**Results of the structures**								
	NH_2-UA	**adenosine**	**Pi**	**CO_2**	**ribose**	**acetic acid**	**Pyru-vate**	**new ADP**	**ATP**
4 Fe_8-S_7	4	4	8	12	4	4	4	4	new 4
4 Fe_2-S_2			16	8					ADP-ATP 20
1 Fe_4-S_4			4	2					
1 Fe_3-S_4			4	2					
ETC	4	4	32	24	4	4	4	4	24

Table II. Results of structures of energy transformations in the electron transfer chain

Abbreviation: NH_2-UA: nitrified uric acid; Pi: PO_3^{3-}

Continuous electron and ATP production, the two steps of ATP production

The process of energy conversion runs in the ETC. The first step produces aminated uric acid, PO_3^{3-} and ADP molecules. Finally, ATP synthase produces ATP from ADP + PO_3^{3-}.

The synchronous activity of the neighbor ETCs in the Multiplex Electron Transfer Chain

The neighboring ETCs (ETC1 - ETC2) operate in synchronization. They mutually activate each other (Table III). The ETC1 and 2 contain 2 x 4 [Fe_8-S_7] clusters. The first [Fe_8-S_7] of the ECT1 is connected to the fourth [Fe_8-S_7] of the ETC2, the second [Fe_8-S_7] of the ECT1 is connected to the third [Fe_8-S_7] of the ETC2, the third [Fe_8-S_7] of the ECT1 is connected to the second [Fe_8-S_7] of the ETC2, while the fourth [Fe_8-S_7] of the ECT1 is connected to the first [Fe_8-S_7] of the ETC2. AA molecules connect the

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neighboring ETCs. One of the ETC pairs is in the clearing stroke, while the other is in the active stroke (Figure 5).

Active stroke Clearing stroke

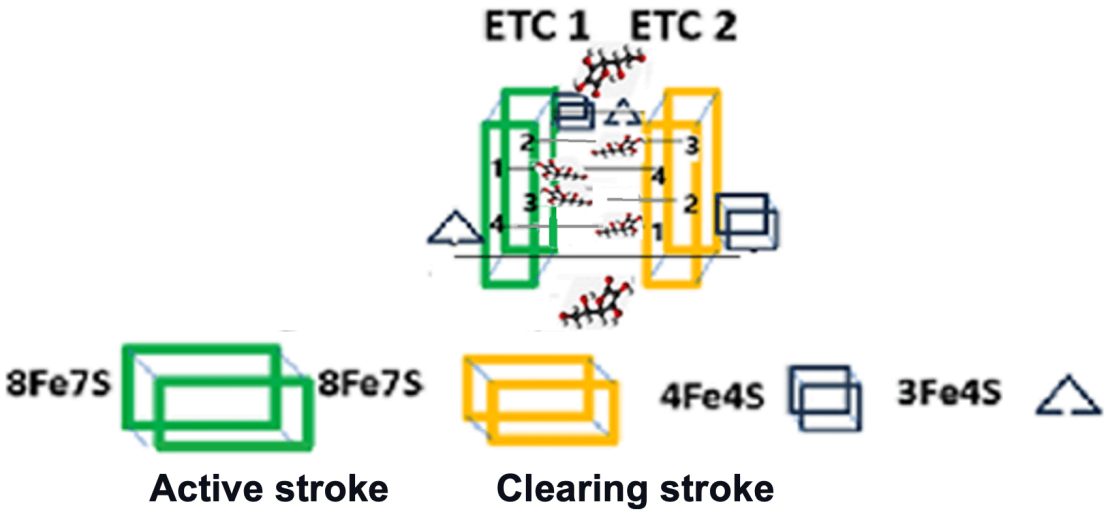

Figure 5. The synchronized activity of the neighboring ETCs

The Structures of Energy Transformation

The Fe-S clusters are linked by cys-S bonds, forming one METC, where the transformation is completed. We suppose that instead of the seven [Fe_4-S_4] clusters proposed by Austin et al.[5] there are four [Fe_8-S_7] clusters of nitrogenase, one [Fe_4-S_4] one [Fe_3-S_4], and 4 [Fe_2-S_2] clusters in the ETC. Two ETCs and ATP synthases form the SET. One [Fe_4-S_4], four [Fe_2-S_2], one [Fe_3-S_4], and four [Fe_8-S_7] clusters are responsible for the production of 32 PO_3^{3-}s (Table II).

Two ETCs and the ATP synthase enzymes could form the METC, the SET-AG, which produces 2 X 4 [ATP, and 24 CO_2] (Table II). The 48 O^{2-} must react with 24 carbons to produce 24 CO_2. The carbon atom of the 24 CO_2 comes from the eight carbon atoms of the eight glucose-ribose conversions and 8 molecules of acetic acid. ATP synthetase and many other specific enzymes are responsible for the proper functioning of METC.

ATP and AA are the determinants of the continuity of the electron transfer. The energy input results in 4 new + 20 ADP molecules. The ADP molecules are transformed into ATP in the ATP synthase using the

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energy gained from the oxidation of 24 carbon atoms before the end of the transformation.

The Structure of Energy Transformation for Aerobic Glycolysis

The SET of Aerobe Glycolysis (SET-AG) consists of two ETCs. Each produces four new ATP. The neighboring ETCs (ETC1 - ETC2) work in synchrony (Figures 5 and 6).

The Structure of Energy Transformation for Oxidative Phosphorylation (SET-OP)

SET-OP has SET-AG + Pyruvate dehydrogenase. SET-OPs are located in the mitochondria.

Hypothesis Explanation

Glucose and ATP are the main sources of energy for cells. During glucose metabolism, ATP is formed in specific cell structures. Eukaryotic cells have the SET-AG and the SET-OP. This enables them to survive in hypoxic–anoxic conditions.

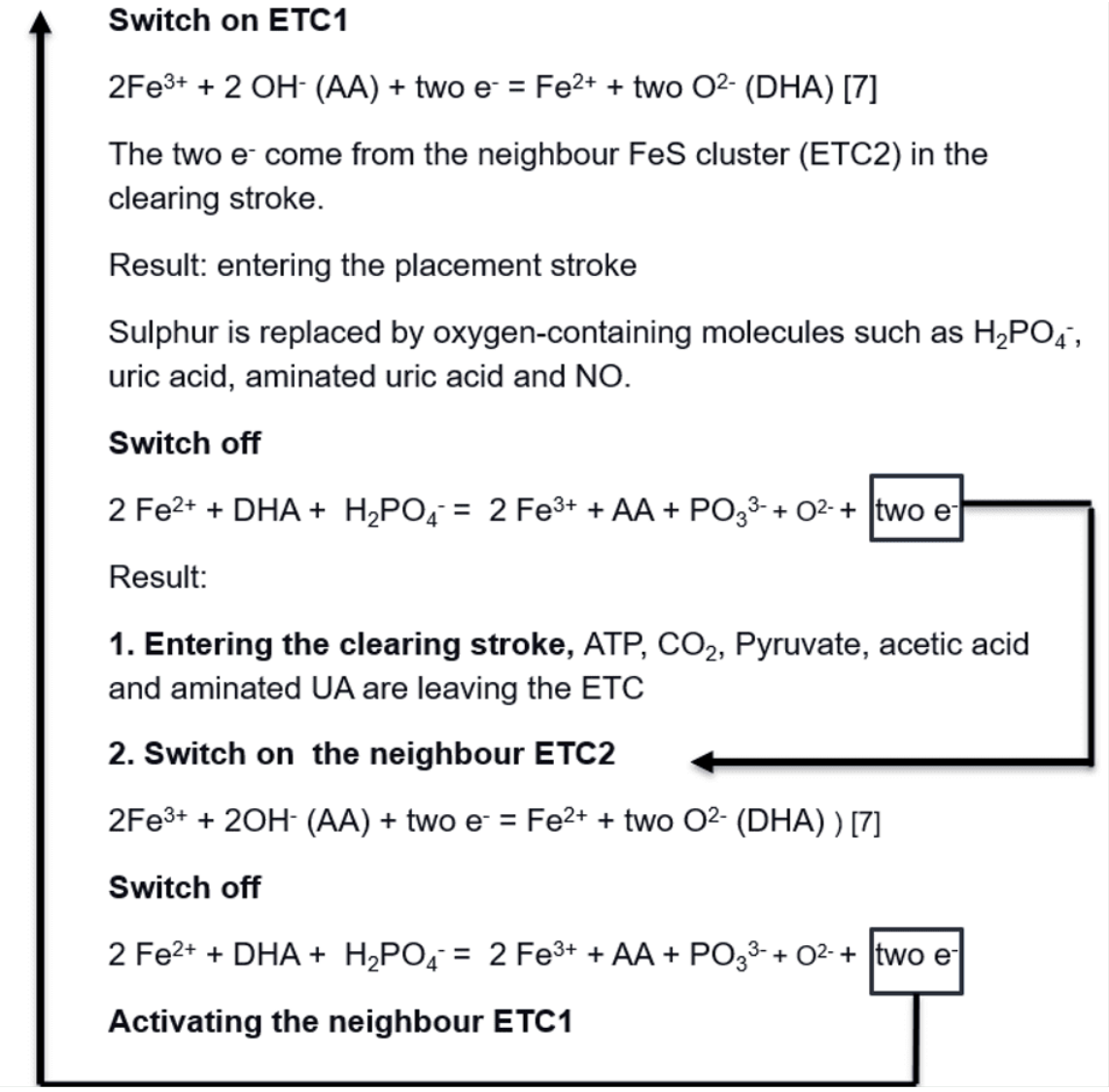

Table III. The synchronized activity of the neighboring ETCs.

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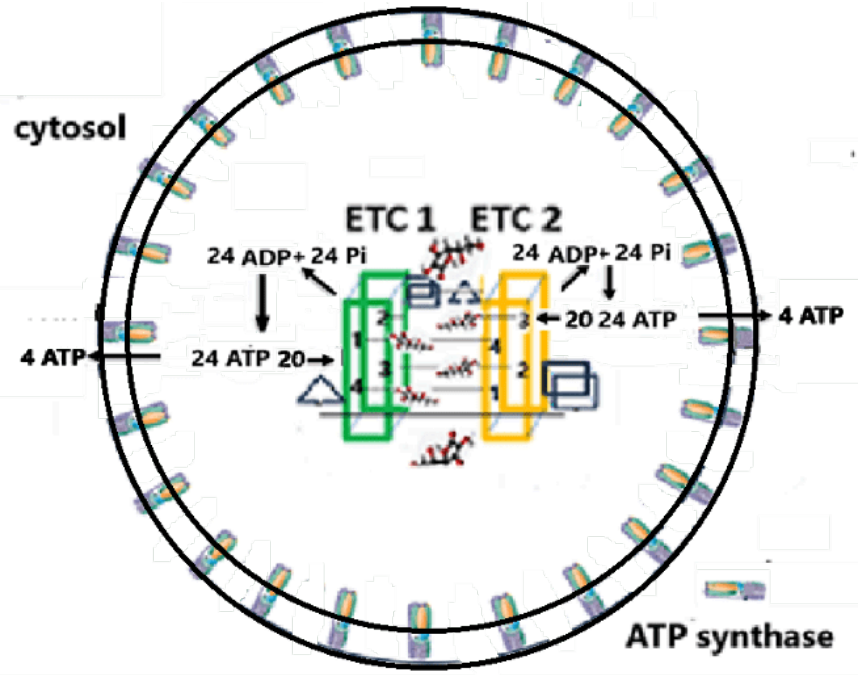

Figure 6. The SET-AG contains two synchronously operating electron transfer chains and 24 ATP synthases.

Abbreviation: Pi = PO_3^{3-}

The summary of the hypothesis:

SET-AG: 2 X [4 UA + 4 NH_2-UA + 4 NO + 32 $H_2PO_4^-$ + 8 D-glucose + 8 acetic acid] =

2 X [4 ATP + 4 NH_2-UA + 4 Pyruvate + 4 acetic acid + **24 CO_2**] + **energy.**

SET-OP in the present of O_2: 2 X [4 UA + 4 NH_2-UA + 4 NO + 32 $H_2PO_4^-$ + 8 D-glucose + 8 acetic acid + 4 Pyruvate] = 2 X [4 ATP + 4 NH_2-UA + 4 acetic acid + 4 Pyruvate + (24 CO_2 + 3x4=12 = **36 CO_2** by pyruvate dehydrogenase)] + **ENERGY** + **defense against free radicals.**

SET-OP without O_2: 2 X [4 UA + 4 NH_2-UA + 4 NO + 32 $H_2PO_4^-$ + 8 D-glucose + 8 acetic acid] = 2 X [4 ATP + 4 NH_2-UA + 4 acetic acid + 4 Pyruvate + 24 CO_2] + **energy**

Energy and CO_2 production in the energy-producing units

Energy is needed to make ATP molecules. Twenty ATP starts the reaction, resulting in 20 ADPs. During the transformation, 24 (SET-AG) or 36 (SET-OP) carbon atoms are oxidized, producing energy. This

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energy, together with the energy of the 20 ATP—ADP conversion, creates four new ATP molecules and regenerates the 20 ADP to ATP molecules.

High-dose vitamin C kills cancer cells.

Cancer cells need 200 times more D-glucose than normal cells. After intravenous AA infusion, the O^{2-} atoms, which are responsible for the oxidation of the carbon atoms, will destroy the cell in case of the absence of the glucose molecule.

Discussion

The similarity between the hypothetical ETC and known structures.

1. Similarities and differences between the hypothetical ETC and the Austin model[5].
    1. The four ADPs originate from two Flavin Adenosine Dinucleotides (FADs) and two Nicotinamide Adenosine Dinucleotides (NADs).
    2. We propose that in complex I, instead of the six [Fe_4-S_4], four [Fe_8-S_7] clusters are responsible for the electron transfer.
    3. We assume that Fe-S clusters are connected to form the continuous METC. The two [Fe_4-S_4] clusters bound the two ETCs via two [Fe_3-S_4] s; thus, the [Fe_4-S_4] and [Fe_3-S_4] clusters correspond to complex II[5].
2. Korth et all suggested that two L-vitamin C molecules are located in the NADPH pocket, presumably near the adenine binding site in the inner membrane of the mitochondria[4].

In our hypothesis, two AAs are located in the first and third [Fe_8-S_7] clusters, close to the adenine bounding site and the nicotinamide (Figure 4).

The hypothesis predicts that ATP and AA initiate the energy transformation, where new ATP, CO_2, H^+, acetic acid, Pyruvate molecules and energy are produced from D-glucose molecules. The construction of the four new ATP molecules needs the energy from twenty ATP molecules. Finally, the 24 of ADP are converted into 24 molecules of ATP using the energy obtained from the oxidation of 24 carbon atoms (eight acetic acids, eight carbon from glycose–ribose conversion). The process of transformation results in all METC producing 48 O^{2-}; which could destroy the cell if the glucose molecules are not

available in the cell. This hypothesis raises the possibility that vitamin C could play a role in cellular energetics, warranting further research in the context of cancer metabolism[18].

This manuscript presents a novel hypothesis regarding ATP synthesis via the Structure for Energy Transformation (SET). While the proposed mechanism integrates known biochemical components, it remains a theoretical model that has not yet been empirically validated. The following limitations should be considered:

1. The SET hypothesis has not been tested through direct biochemical assays or in vivo experiments.
2. The role of Fe-S clusters, uric acid, and vitamin C in ATP synthesis within this framework is speculative and requires further experimental confirmation.
3. Although the hypothesis suggests a potential link between vitamin C metabolism and cancer treatment, no clinical studies have directly tested this mechanism.

Future research should aim to experimentally validate the SET model and assess its physiological relevance before clinical applications are considered.

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