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The Dual Energy Supply of Eukaryotic Cells

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Summary

The regeneration of tissue damage is possible because our cells have a dual-energy supply system and can ensure tissue regeneration without O2. The publication summarizes the defining elements of the structures responsible for energy and energy-carrier transformation, specifically, the hypothetical Adenosine Diphosphate (ADP)-producing unit, the Structure for Energy Transformation (SET) of anaerobic glycolysis (SET-AG), and the SET of oxidative phosphorylation (SET-OP). SET-AG is responsible for the anaerobic fermentation, while SET-OP is for the aerobic oxidative phosphorylation. The hypothesis also describes where the ADP, PO33- and energy for ATP formation come from during ATP synthesis in ATP synthase.

Keywords: Eukaryotic Cell; Hif; Cell Energetic; Tissue Regeneration; Fe-S Cluster.

Energy Conversion

Gasoline or petrol, used as a fuel in spark-ignited internal petrol engines, must be made by fractional distillation of petroleum. Similarly, glucose must be transformed into Adenosine Triphosphates (ATP) for living organisms to get a usable energy carrier. The human body comprises eukaryotic cells, so it is essential to know the properties of their energy supply. This communication summarizes the evolution of eukaryote cells and their energy supply path — the dual energetic stock results in the possibility of the regeneration of tissue damage.

The Hypothetical Way of The Energy and Energy-Carrier Transformation, ATP Synthesis

Glycolysis and oxidative phosphorylation are autonomous mechanisms. It is well known that the energy supply of cells is provided by glycolysis which occurs in the cytosol of cells. During glycolysis, glucose breaks down into Pyruvate and energy; 2 ATP is derived: Glucose + 2 Nikotinamid-adenin-dinucleotide (NAD+) + 2 ADP + 2 Pi → 2 Pyruvate + 2 NADH + 2 H+ + 2 ATP + 2 H_2O. The specific form of glucose used in glycolysis is glucose 6-phosphate. Under aerobic conditions, Pyruvate derived from glucose will enter the mitochondria to undergo oxidative phosphorylation. Anaerobic conditions result in Pyruvate staying in the cytoplasm and being converted to lactate by the enzyme lactate dehydrogenase [1, 2].

Energy is liberated in the cells during energy transformation. At the same time, ATP, one new energy-carrier molecule, will be created. We suppose that a hypothetical structure is responsible for ADP production. Based on this hypothesis, it is proposed that glucose, NH3, uric acid, and H2PO4- will result in the formation of ATP. In addition, ribose, the part of the adenosine + CO2, Pyruvate, and acetic acid, will be created from the D-Glucose during the process. Energy and energy-carrier transformation are realized in unique permanent structures such as Structure for Energy Transformation (SET). The Connecting Unit 1 (CU1), Adenosine Diphosphate Producing Unit (ADP-PU), Pi- Producing Unit (Pi-PU) and Connecting Unit 2 (CU2), are the basic units of SETs. The SET of anaerobic glycolysis (SET-AG) is responsible for the anaerobic fermentation, while the SET of oxidative phosphorylation (SET-OP) is for the aerobic oxidative phosphorylation.

The Development of Eukaryotic Cells

There was no O_2 in Earth's atmosphere more than three billion years ago. At that time, the possibility of the formation of life was already ensured. One of the earliest cells to produce oxygen were the cyanobacteria (blue-green algae), which evolved oxygen via photosynthesis. The appearance of O_2 in the atmosphere caused the first environmental disaster, as the ancient fermenting microorganisms did not have sufficient defense capacity against the highly destructive O_2.

According to Lynn Margilus' hypothesis, an ancient cell entered into symbiosis with a cell that could defend itself against the dangerous effects of O_2 (Illustration 1). In addition, the modern cell produced an order of magnitude more energy with the help of O2. The contemporary organelle is now known as a mitochondrion [3].

The Evidence Supporting the Endosymbiotic Conception: [3, 4]
a/ Mitochondria are capable of division, and their dimensions and form are like today's bacteria.
b/ They have their DNA, which is identical in structure to the DNA of prokaryotes.
c/ They have a protein-synthesizing system, similar to prokaryotes.
The advantages of symbiosis are: significantly more energy, protection against free radicals, and the regeneration ability of organisms [3, 4].

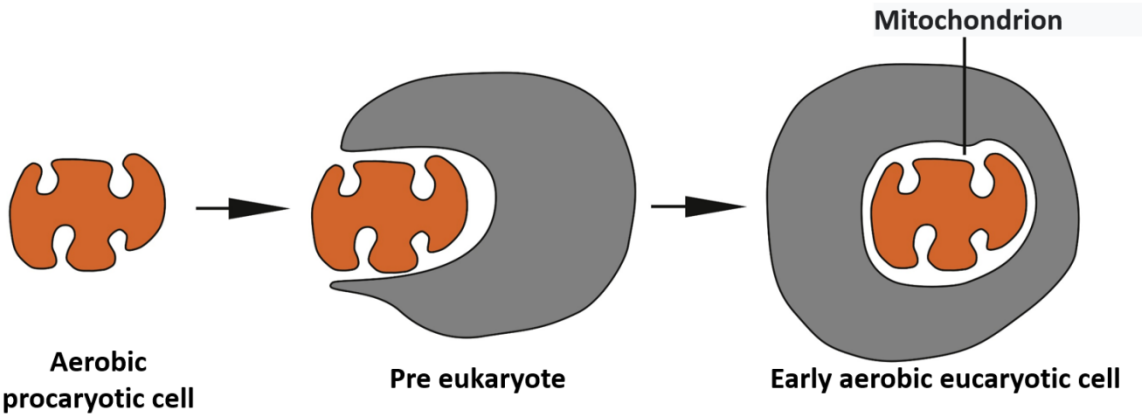


Illustration 1: The procaryotic cell, which developed features of an early mitochondrion (defense system against reactive oxidative species and aerobic energy production), fuses with pre-eukaryote to give rise to an early aerobic eukaryotic cell. [3]

The Peroxisome

Peroxisome is a membrane-bound oxidative organelle, a type of micro-body, found in the cytoplasm of virtually all eukaryotic cells [5-7]. They perform critical roles in lipid metabolism and the conversion of reactive oxygen species. They also contain approximately 10% of the total activity of two enzymes (Glucose-6-phosphate dehydrogenase and 6-Phosphogluconate dehydrogenase) in the pentose phosphate pathway [8]. Which is essential for energy metabolism [9]. Key players in peroxisome division are conserved in animals, plants, and fungi, and key fission components are shared with mitochondria [10].

The Electron Transport Chain

An electron transport chain (ETC) is a series of protein complexes and other molecules that transfer electrons from electron donors to electron acceptors via redox reactions (both reduction and oxidation co-occurring) and couples this electron transfer with the transfer of protons (H+ ions) across a membrane. The electrons transferred to the ETC involve four multi-subunit large enzyme complexes and two mobile electron carriers. Many of the enzymes in the electron transport chain are membrane-bound [11, 12].

Regulation by the HIF System, The Control of Tissue Regeneration

Cells will become viable in a hypoxic environment with the help of the Hypoxia-Induced Factor (HIF) system, which ensures adaptation to a hypoxic environment. The HIF-1α subunits are continuously synthesized and degraded under normoxic conditions, while it accumulates rapidly following exposure to low oxygen tensions. Thus, due to the lack of O_2 caused by injury or any reason, the hydrolysis of the HIF-1α is annulled [13,14].

HIF-1 α combines with HIF-1 beta to modify the activity of about 200 genes. As a result, the circulation will be restored with the help of newly formed blood vessels. After that, increasing tissue O_2 will hydrolyse the HIF-1α; thus, the cells will return to the mitochondrial oxidative phosphorylation. [14, 15].
The most significant changes are: [6, 7, 16, 17]

- Due to the low energetic efficiency of SET-AG, the appropriate energy supply of the cell can be realized only by about two hundred times more glucose. Therefore, the number of glucose transporters of the cells increases.
- The sensitivity to apoptosis decreases.
- Induction of neovascularization.
- Induction of the formation of pluripotent cells.

As a result of these changes, the cells survive in the hypoxic environment and ensure the realization of tissue regeneration and neovascularisation (Illustration 2). [6, 7, 16, 17]

The Dual Energy Supply of Eukaryotic Cells

Eukaryotic cells have two genetic stocks, as mitochondria contain their own. Accordingly, our cells must have two structures to ensure energy and energy-carrier transformation. SET-AG (belonging to the ancestral cell) and SET-OP (belonging to the mitochondria). The operational activity of these structures can be determined by the amount of ATP produced.

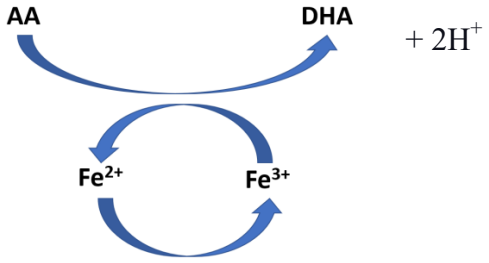
In an anoxic or hypoxic environment, the High-molecular weight cytochrome (HMWC) part of the mitochondria stops working. At the same time, there is no hydrolysis of HIF-1 α, which will result in the tissue regeneration (Illustration 2).

Abbreviations: SET-AG: Structure for Energy Transformation of Anaerobic Glycolysis; SET-OP: Structure for Energy Transformation of Oxidative Phosphorylation; HMWC: High-molecular weight cytochrome; HIF: Hypoxia-Inducible Factor.

Illustration 2: The HIF system is the detector and organizer of the oxygenated and O_2-free environment.

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

Kinga Linowiecka et al. stated that ascorbic acid (AA) is an oxidative stress sensor and a gene expression regulator. In addition, they pointed out that the change of AA to dehydroascorbic acid (DHA) regulates the modulation of the iron's electron state in Fe^{2+} -dependent dioxygenases (Illustration 3). [18]. Two protons (H+) are liberated during the AA – DHA transformation.



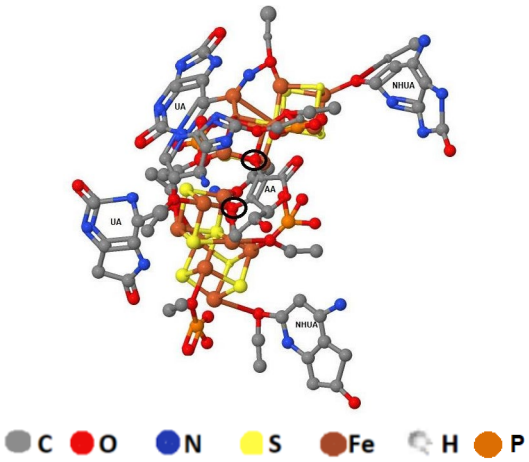
Abbreviations: AA: ascorbic acid; DHA: dehydroascorbic acid

Illustration 3: Vitamin C's role in Fe^{3+} - Fe^{2+} transformation. [18]

This change might also be valid for the Fe-S clusters' Fe atoms. The reaction results in a sulphur-oxygen exchange, creating four O^{2-} in the 2Fe-2S cluster.

A similar reaction might occur by the two OH of the ribose part of the ATP activating the Fe-S cluster.

Illustration 4. presents the bound of two 8Fe-7S clusters by vitamin C (circle).

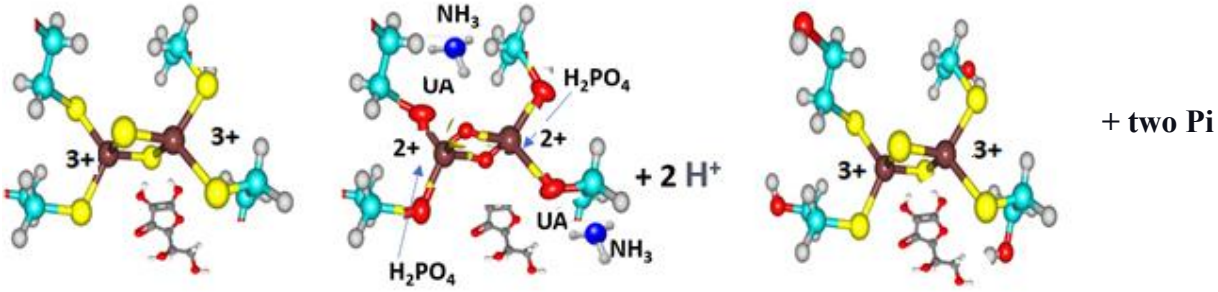


Abbreviations: UA: Uric Acid, NHUA: Aminated Uric Acid, AA: Ascorbic Acid

Illustration 4: One Ascorbic Acid bounds two 8Fe-7S clusters by its two OHs of the lactone ring.

The importance of Fe-S clusters

Several Fe-S clusters [e.g.,2Fe-2S(cys-S)$_4$, 3Fe-4S(cys-S)$_3$, 4Fe-4S(cys-S)$_4$, P-cluster of nitrogenase 8Fe-7S (cys-S)$_6$] are known. They play an essential role in maintaining life by ensuring continuous electron transfer. In the central part of the 2Fe-2S cluster, two irons are bonded to two sulphurs. The two irons in the 2Fe-2S cluster can bind four more sulphurs. The iron of Fe-S clusters is Fe^{2+}, or Fe^{3+} forms. The number of electrons in the outer electron shell influences the iron's binding affinity to oxygen and sulphur. In the case of Fe^{3+}, it binds the sulphur, while in the case of Fe^{2+}, the oxygen bind is preferred (Illustration 5) [19, 20].



AA DHA + H^+ AA

Abbreviations: AA: ascorbic acid; UA: uric acid; DHA: Dehydro ascorbic acid.

Illustration 5: The two oxidation states of 2Fe-2S cluster. Other Fe-S clusters might have similar nature. Thus, the 8Fe-7S P-cluster of nitrogenase has six Cys-S structures [19] and might produce six O^{2-}(illustration 6).

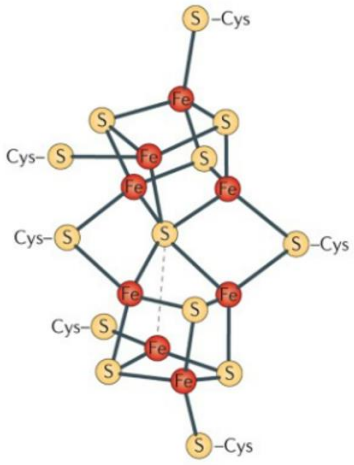


Illustration 6: 8Fe-7S (cys-S)$_6$ P-cluster of nitrogenase[21].

Fe^{3+} modification to Fe^{2+} results in the possibility of binding oxygen-containing molecules, such as H_2PO_4-, uric acid (UA), or aminated UA. Then, in an additional step, Fe^{2+} returns to Fe^{3+}. The change results in three O^{2-}production in the 3Fe-4S cluster, four in the 2Fe-2S and 4Fe-4S clusters, and six O^{2-} in the 8Fe-7S cluster. All Fe-S clusters produce O^{2-}. It must result H_2O or react with carbon, producing CO_2. As the structures of the Fe-C clusters are known, the carbon sources (D-Glucose, Pyruvate, or acetic acid) can be calculated (Table I).

The Functional Importance of Fe-S clusters' s cys-S Components.

The cys-S components of the Fe-S clusters (R-$SCH_2CH(NH_2)CO_2H$) contain one sulfur atom, one carboxamide, one carboxyl part, and one OH (Illustration 7).

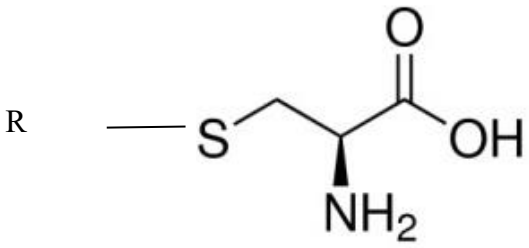


Illustration 7: The structure of cys-S.

NH_2 part of the cys-S

The NH_2 part of the structure might bind D glucose (Illustration 8) or L ascorbic acid molecules.

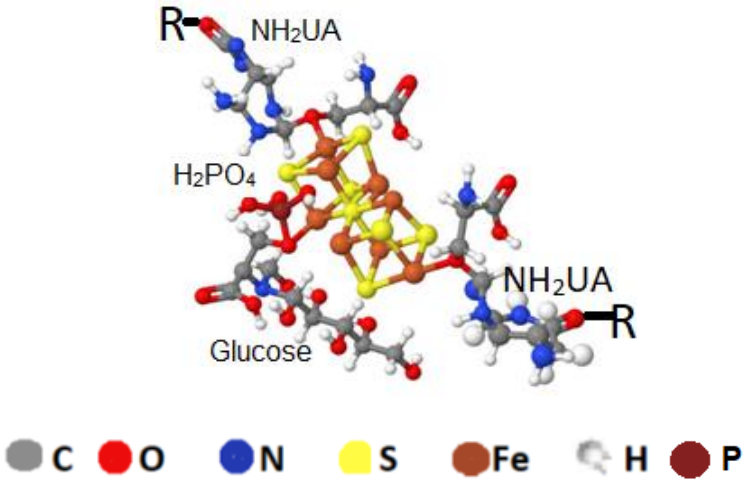


Illustration 8: 8Fe-7S(cys-S)6, glucose, H_2PO_4-, two NH_2uric acids (NH_2UA). (Only three cys-S are illustrated).

Carboxyl part of the cys-S

The C=O part might bind the adenine of ATP (Illustration 9). Circle 1 indicates the binding of NH_2 to the oxygen, while circle 2 demonstrates the change of sulfur atoms to oxygen by the two OH of the ribose belonging to ATP.

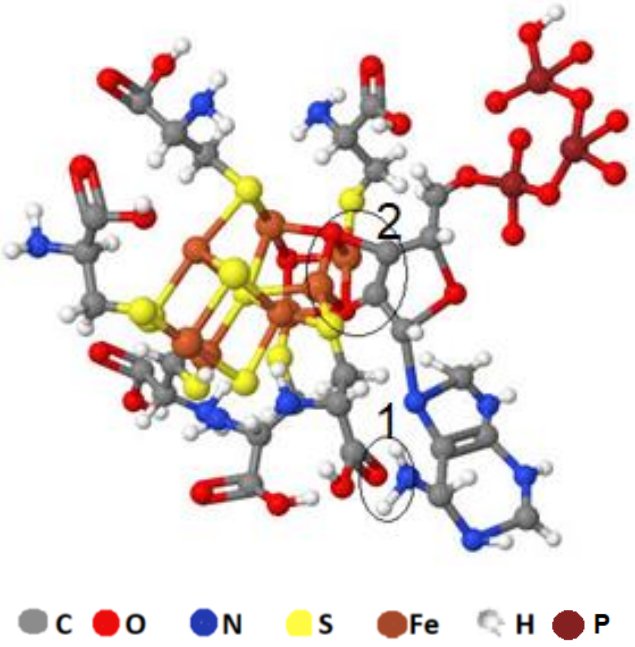


Illustration 9: One ATP binds to 8Fe-7S(cys-S)6 cluster.

Carbamide – Carboxyl Connection between Two Cys-S Chains

Fe-S clusters might create one Multipart Electron Transfer Chain (METC) realized by the cys-S parts of the clusters. The NH_2 and the Carboxyl parts of the cys-S offer the possibility of continuous chain creation, as demonstrated in Illustration 10.

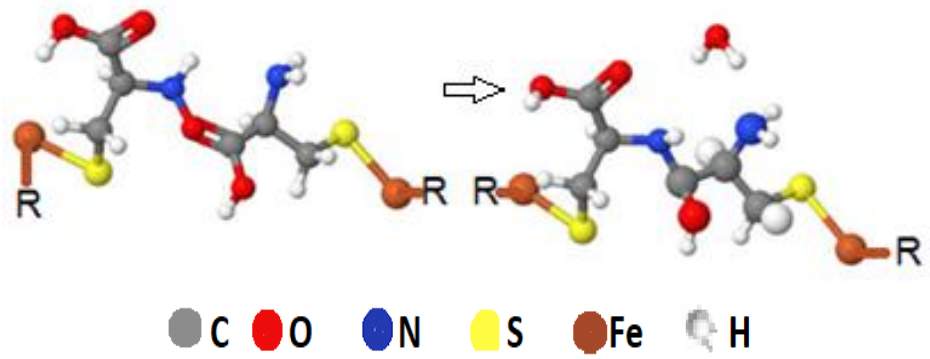


Illustration 10: Two cys-S chains are connected with the help of the carbamide and carboxyl parts of the structures while liberating one H_2O.

Transformation of the source molecules.

NH_3 Uric Acid – NHUA transformation in the ADP-PU.

One O^{2-} transforms NH_3 + one uric acid (UA) in the 8Fe-7S cluster of nitrogenase.

$UA + NH_3 + O^{2-} = NHUA + H_2O$

D-glucose

Glucose – ribose – Pyruvate - acetic acid transformation in the ADP-PU.

Ribose + CO_2 is created from D-glucose during the transformation in the ADP-PU.

D glucose + two O^{2-} = D-ribose + CO_2 + energy

Ribose + adenine = adenosine

Ribose is transformed into Pyruvate and acetic acid.

Acetic acid + four O^{2-} = two CO_2 + two H_2O + energy

Pyruvate + six O^{2-} = three CO_2 + three H_2O + energy

H_2PO^{4-}

Dihydrogen phosphate will be transformed into PO_3^{3-} (Pi) + two $H^+ + O^{2-}$ in the Pi-PU (16), the ADP-PU (12), CU1(3) and the CU2 (4).

ATP synthase – Complex V

The binding change mechanism of ATP synthase involves the active site of a β subunit's cycling between three states. [22] In the "open" state, ADP and Pi enter ATP synthase. The enzyme then changes shape and forces these molecules together, with the active site in the resulting "tight" state binding the newly produced ATP molecule. Finally, the active site cycles to the loose state and will be ready for the next cycle of ATP production. [22, 23]

Structures for energy and energy-carrier transformation

SETs are places of energy and energy-carrier transformation. They contain permanent structures where the arriving source molecules are converted to energy, new energy-carrier molecules (ATP), and CO_2.

The transformation is completed in an electron transfer structure. Four 2Fe-2S, one 3Fe-4S, and seven 4Fe-4S clusters offer the proper function of the complex, as described by Austin et al. [24] (Illustration 11).

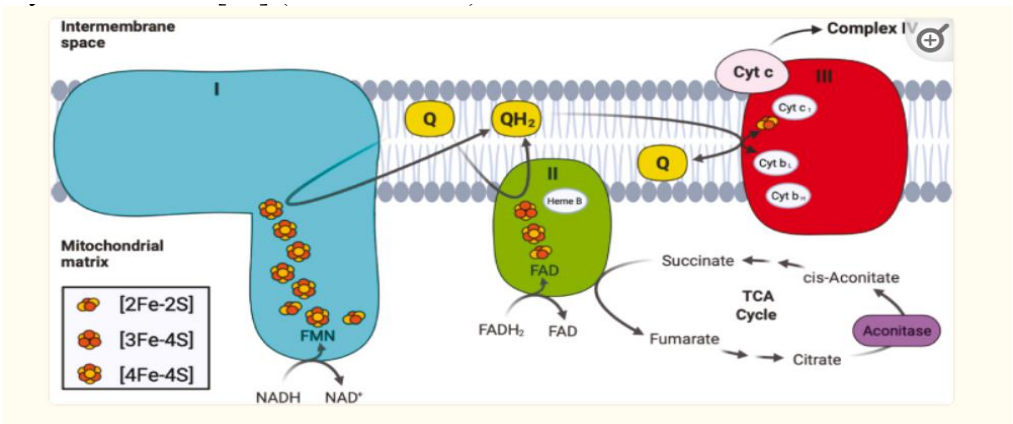


Illustration 11: A simplified version of the mitochondrial ETC, showing Complexes I (blue), II (green), and III (red). [24]

The Hypothetical Way of the Energy Transformation

The hypothesis of Multipart Electron Transfer Chain

The METC might contain one 4Fe-4S cluster and four 8Fe-7S clusters of nitrogenase instead of the seven 4Fe-4S clusters, as suggested by Austin et al. [22]. The four 8Fe-7S clusters of nitrogenase are the determining part of the ADP-PU. The remaining 4Fe-4S cluster, the CU2, might be responsible for connecting the 6 METS, while the four 2Fe-2S might create the Pi-producing Unit (Pi-PU). The units are connected by carbamide carboxyl bound, creating the METC.

The change of Fe^{3+} to Fe^{2+}

The two OH of AA on the lactone rings (Illustration 6) and the two OH of the ATP's ribose (Illustration 9) change the nature of the Fe atoms from Fe^{3} to Fe^{2+}. [18].

SET is activated by ATP and initiated by AA. Their ratio determines the initiation. A high intracellular AA level increases the activity of SET, and a high ATP level decreases it.

Building units of METC

Connecting Unit 1 (CU1)

Molecule of the permanent structure:

One 3Fe-4S $(cys\text{-}S)_3$ cluster (Illustration 12).

Two CU1s of two METCs are responsible for the oxidation of one Pyruvate molecule.

Source molecules **of two CU1**:

2 X Three H_2PO^{4-} + one Pyruvate

Products of two CU1s:

2 X [Three PO_3^{3-} (Pi) + six H+] + 3 CO_2 + energy

Activation of the Connecting Unit 1

Two ATP, forming two glucose 6 phosphate (G6P)s, activate and two AA initiates two CU1s.

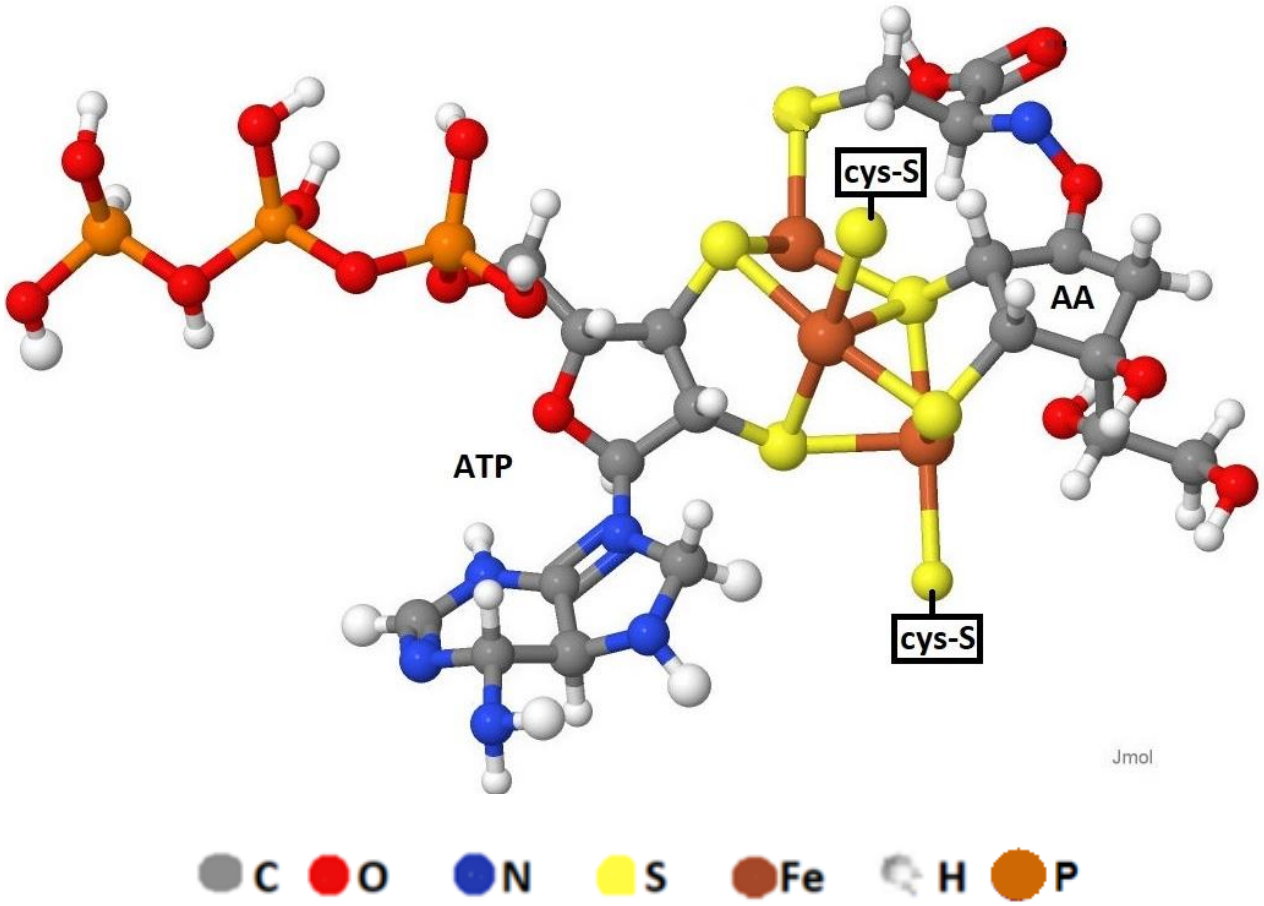
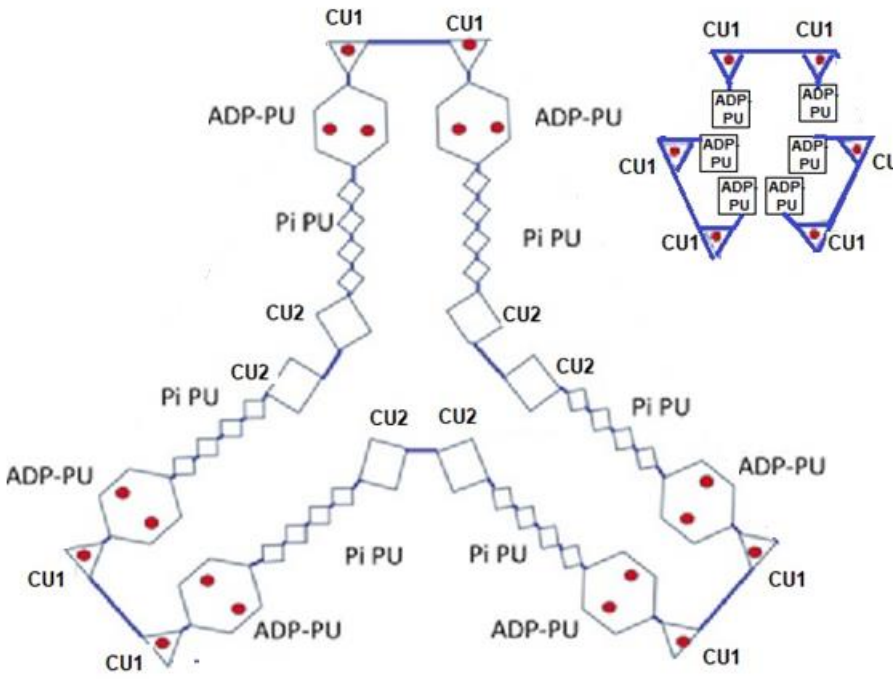


Illustration 12: Connecting Unit 1. Two OH of Ascorbic Acid (AA) activates, and two OH of vitamin C initiate the transformation process.

The six CU1s and the six CU2s connect the six METC of the SET-AG, creating a continuous connection between all units of the SET-AG (Illustration 13).



Abbreviations: CU1: Connecting Unit 1; ADP-PU: ADP Producing Unit; Pi-PU: Pi-producing; CU2: Connecting Unit 2.
Illustration 13: The Connecting Unit 1 and Connecting Unit 2 connect the six METCs points of initiation by vitamin C.

PO_3^{3-} (Pi) Producing Unit (Pi-PU)
Molecules of the permanent structure:
Four 2Fe-2S $(cys\text{-}S)^4$ clusters.

Source molecules:

4 x [acetic acid+ four H_2PO^{4-}]

Products:

4 x [four Pi + 8 H+ + two CO_2 + energy].

Activation of the unit
Four ATP are responsible for the activation of the structure. **forming four G6P**

Adenosine diphosphate-producing unit

The basic unit of SET-AG and SET-OP is the ADP-PU. In addition, ATP synthase is also required to generate ATP.

Molecules of the Permanent Structure:
Four 8Fe-7S $(cys\text{-}S)_6$ P clusters of nitrogenase, one Flavin, and one nicotinamide molecule (Illustration 14).
Three ADP-PUs of three METCs are responsible for the oxidation of two Pyruvate molecules.

Source molecules of three ADP-PUs
3 X [Four UA, four NH_2-UA, four NH_3, twelve H_2PO^{4-}, eight D-glucose] + two Pyruvate.
The four NH_2-UA and eight H_2PO^{4-} molecules create the tetra adenine octo phosphate ring, where four 8Fe-7S P-clusters of nitrogenase connect the molecules (Illustration 14).
Products of three ADP-PUs:

3 X [4 ADP + 8 CO_2 + 24 H+ + 4 ribose (4 acetic acid + 4 Pyruvate), 4 Pi] + six CO_2 + energy. The four ADP and four Pi will form four ATP in the ATP synthase.

Connecting Unit 2

Molecule of the permanent structure:
One 4Fe-4S $(cys\text{-}S)_4$ cluster.
Three CU2s of three METCs are responsible for the oxidation of two Pyruvate molecules.

Source molecules of three CU2s:

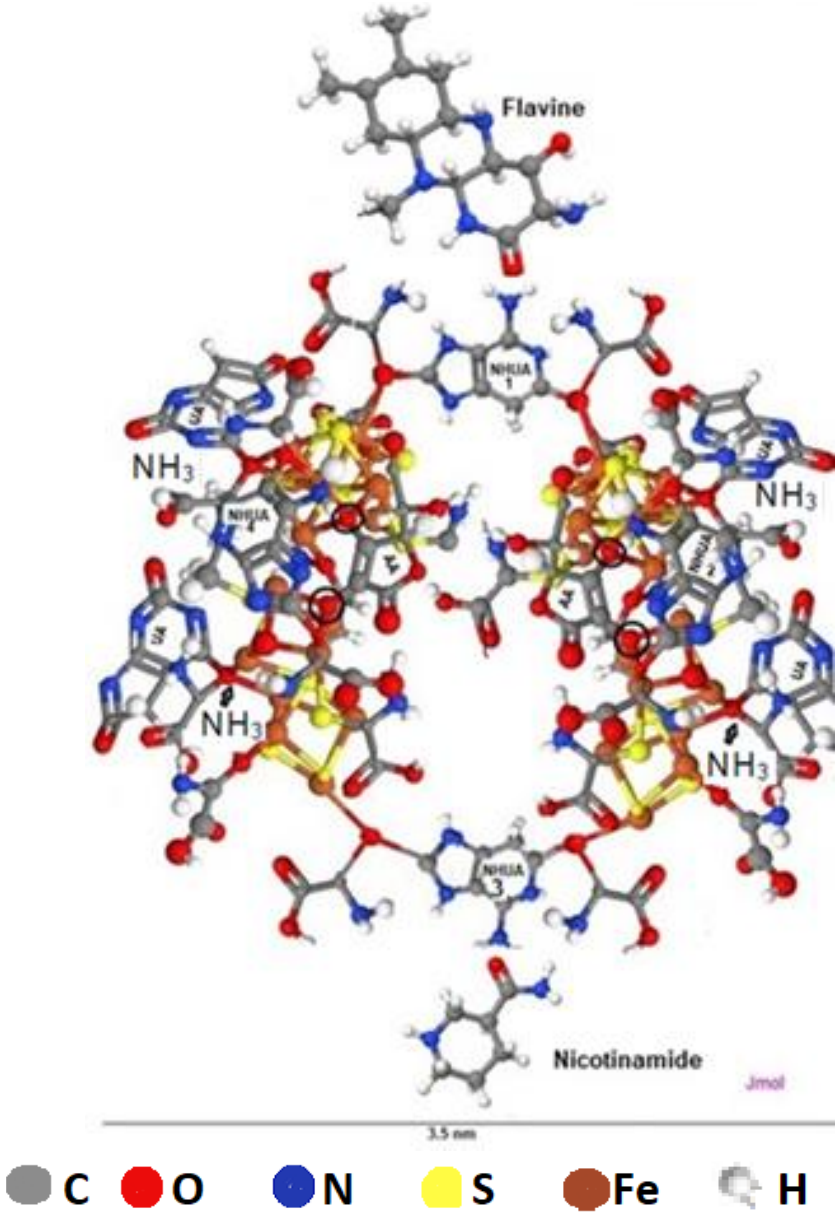
3 X Four H_2PO^{4-} + two Pyruvate

Products of three CU2s:

3 X 4 Pi + six CO_2 + 24 H+ + energy

Activation of the unit
Two ATP are responsible for the activation of one CU2, forming one G6P.

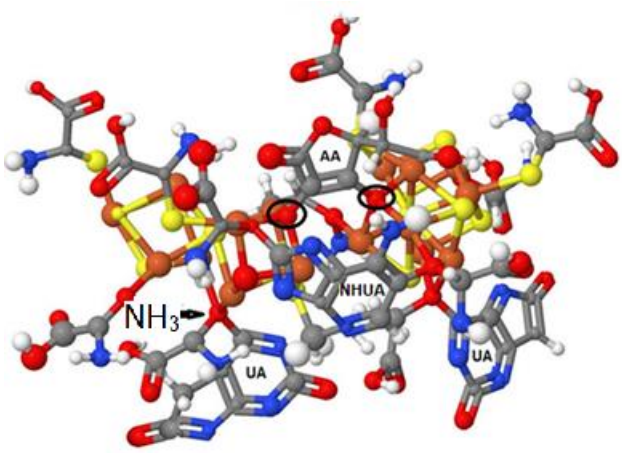
All SETs are built up by Connecting Unit 1 (CU1), PO_3^{3-} (Pi)-Producing Unit (Pi-PU), ADP-PU, Connecting Unit 2 (CU2), and ATP-synthase. Source molecules that arrive at the structure will be transformed into new energy-carrier molecules, CO_2 and energy, while the membrane potential is also realized by protons (H^+).



Abbreviations: UA: Uric Acid; AA: ascorbic acid; The structure's eight D-glucose, and the twelve H_2PO_4 molecules, are not presented.
Illustration 14: Adenosine diphosphate producing unit. Four Fe8-S7 (cys-S)6 clusters of nitrogenase, one Flavine, one Nicotinamide, four UA, four NH_2-UA, and four NH3, in the ADP-PU.

Energy investment: the activation of the four Fe8-S7 (cys-S)6 P-clusters is realized by 12 ATP resulting in 12 ADP. The mechanism of S – O exchange might be similar to the processes of 2Fe-2S as presented above (Illustration 5). The size of the ADP-PU is about 2.5 - 3.5 nm.

The initiation of the four P-clusters 8Fe-7S$(cys\text{-}S)_6$ is realized by two AA molecules. One AA molecule bounds two 8Fe-7S clusters by the seventh sulphur atoms of the two clusters (Illustration 15).



Abbreviations: AA: ascorbic Acid, UA: Uric Acid.

Illustration 15: One ascorbic acid connects two 8Fe7S clusters initiating the electron transfer.

The UA molecule will be aminated by one NH_3 molecule.

The transformation in the ADP-PU results in four ATP, and the nitrification of four uric acids.

SET-AG consists of two x three ADP-PUs (ADP-PU-A, ADP-PU-B, and ADP-PU-C) and six ATP synthases. These structures work together in a synchronized way. Wen ADP-PU releases the ADP and PO_3^{3-}, the ATP synthase is in the open phase, ready to accept them. Furthermore, when ADP-PU-A is in the open state, ADP-PU-B is in the tight, and ADP-PU-C is in the loose state. This synchronization ensures continuous membrane potential and ATP formation (Illustration 16).

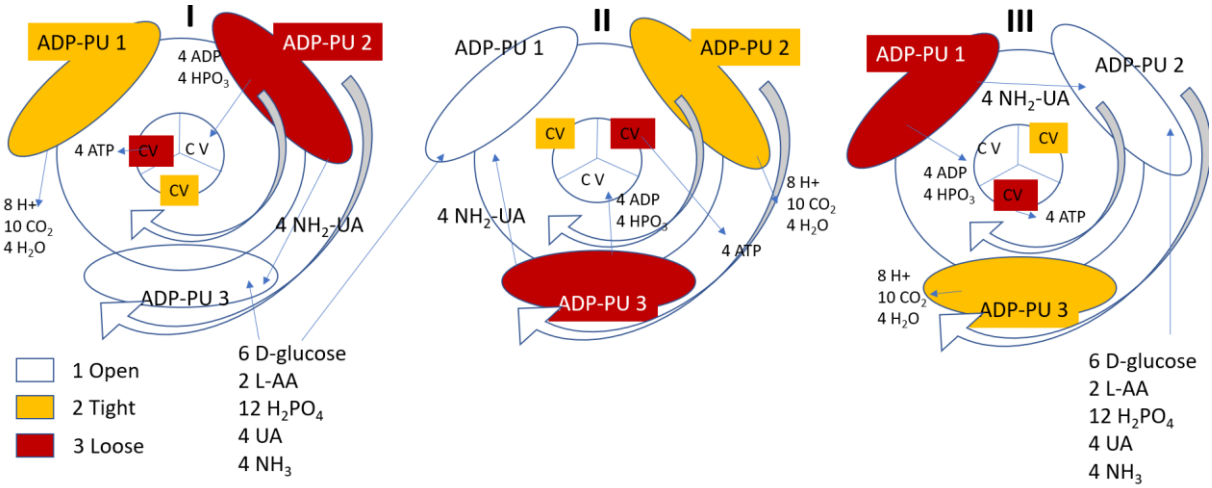


Illustration 16: The synchronised function of three ADP-PUs and three ATP synthases (Complex V: CV)

The four UAs with four NH_3 molecules form four aminated UAs + four H_2O, while the four aminated UAs produce four adenine molecules.

Eight CO_2, four ADP, and four ribose = four Pyruvate + four citric acids are created from eight D-glucose molecules in the transformation process.

Four ribose with four UA-originated adenine molecules forms four adenosines. In an O_2 -free environment, four lactates are formed from the four Pyruvates, while in an oxygenated environment, 4x3 CO_2 molecules + energy are realized through oxidative phosphorylation.

SET-AG has 2 x 3 = 6 METC, each producing 24-11 = 13 Pyruvate + 129 CO_2 + energy.

SET-OP has one SET-AG: (6 METC) + two High Molecular Weight Cytochrome Cs, producing 129 + 12x3 = 36 =165 CO_2 + ENERGY + one Pyruvate.

SET-AG is always present in the cell. They function in normoxic conditions as well. The Pyruvate produced by them forms lactates in the cells.

Sulfur – Oxygen change

The affinity of Fe^{2+} to OH is more extensive than to S.

Binding OH by Fe^{2+} results in three electrons (Fe^{3+}).

Fe^{3+} will become Fe^{2+} by the hydrogen atoms of the H_2PO^{4-} molecules.

AA and ATP can change the S to OH in the Fe-S clusters. First, ATP activates the Fe-S clusters, by two OH and energy transfer. After this, the cluster is ready for function. AA is needed for the initiation, realized by the double bound of the lactone ring of AA. The four Fe8-S7(cys-S)$_6$ clusters of the ADP-PU have 4 x 6 cys-S parts. Thus, they offer places for 24 oxygen-containing molecules as twelve H_2PO^{4-}, four UA, and eight oxygen of four NH-UA molecules. One UA offers one, while the NH_2UA offers two Oxygens. (Illustrations 17). Four similar structures form tetra adenin ring.

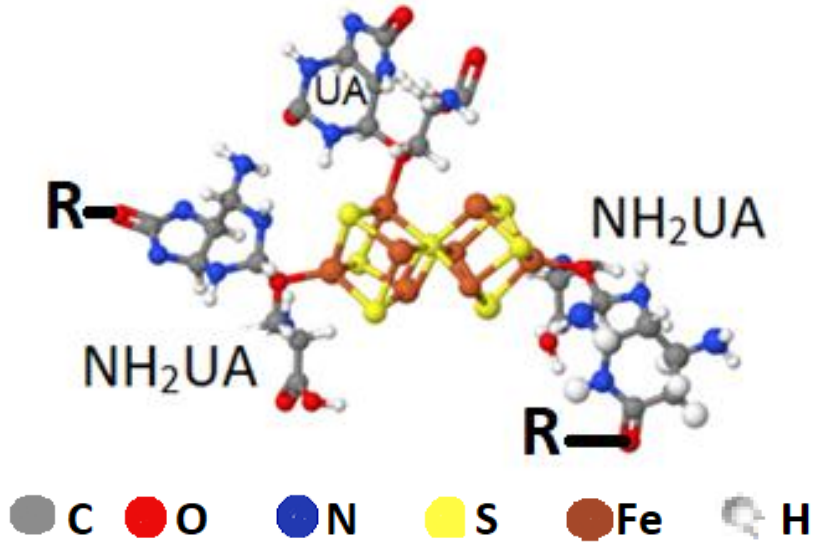


Illustration 17: Uric acid (UA), and two NH_2uric acids (NH_2UA) molecules bounded to 8Fe-7S(cys-S)$_6$ cluster (Only three of the six cys-S are illustrated).

8Fe-7S(cys-S)$_6$

The NH_2 and C=O structures of the cys-S offer connecting points for the stabilization of the complex structure of the METSs. Illustration 18a demonstrates two 8Fe-7S clusters bounded by two cys-S. Illustration 18b shows one NH_2UA molecule attached to the structure.

The four UAs with four NH_3 molecules form four aminated UAs, while the four aminated UAs produce four adenine molecules. Eight ribose molecules are created from eight D-glucose molecules in the transformation process.

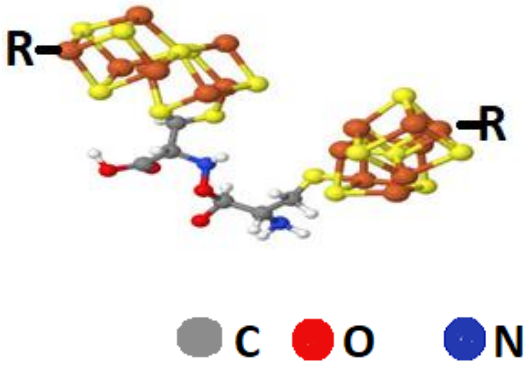


Illustration 18a: Two Fe8-S7 clusters bounded by two cys-S waiting for the NH_2UA.

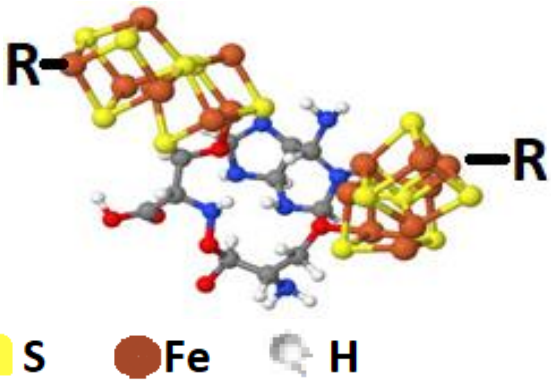


Illustration 18b: NH_2UA molecule attached to the structure.

Four ribose with four NH-UA-originated adenine molecules forms four adenosines, while from the remained four ribose, four Pyruvate, and four acetic acid molecules are formed. Four lactates are formed in an O_2-free environment from the four Pyruvates, while in an oxygenated environment, 4x3 CO_2 + twelve H_2O molecules + energy are realized through oxidative phosphorylation. During the energy transformation, the carbon atoms of the four acetic acids are converted into eight CO_2 in the METC's Pi-PUs of SET-AG and SET-OP.

Structure for anaerobe glycolysis

SET-AG contains six METCs. They produce 24 (6x4) Pyruvate.

The Pyruvate is converted to lactate by the enzyme lactate dehydrogenase. [1, 2] Three of the Pyruvate molecules are converted to nine CO_2 in the six CU1s, while four other Pyruvates are oxidised in the six CU2s, resulting in 12 CO_2 molecules. Four further Pyruvate are oxidised in the six ADP-PUs. The remaining thirteen Pyruvate (24 – (3+4+4) = 13) will be converted to lactate by the enzyme lactate dehydrogenase.

Structure for oxidative phosphorylation

The HMWC can oxidise six Pyruvate (Illustration 19).

The SET-OP consists of six METCs + two MHWCs s oxidizing 12 of the 13 Pyruvate molecules.

In an anoxic or hypoxic environment, the HMWC is not working (Illustration 2). resulting in less energy, as Pyruvate molecules are not oxidised. A further vital consequence of anoxia/hypoxia is that the defence against free radicals is not realized either.

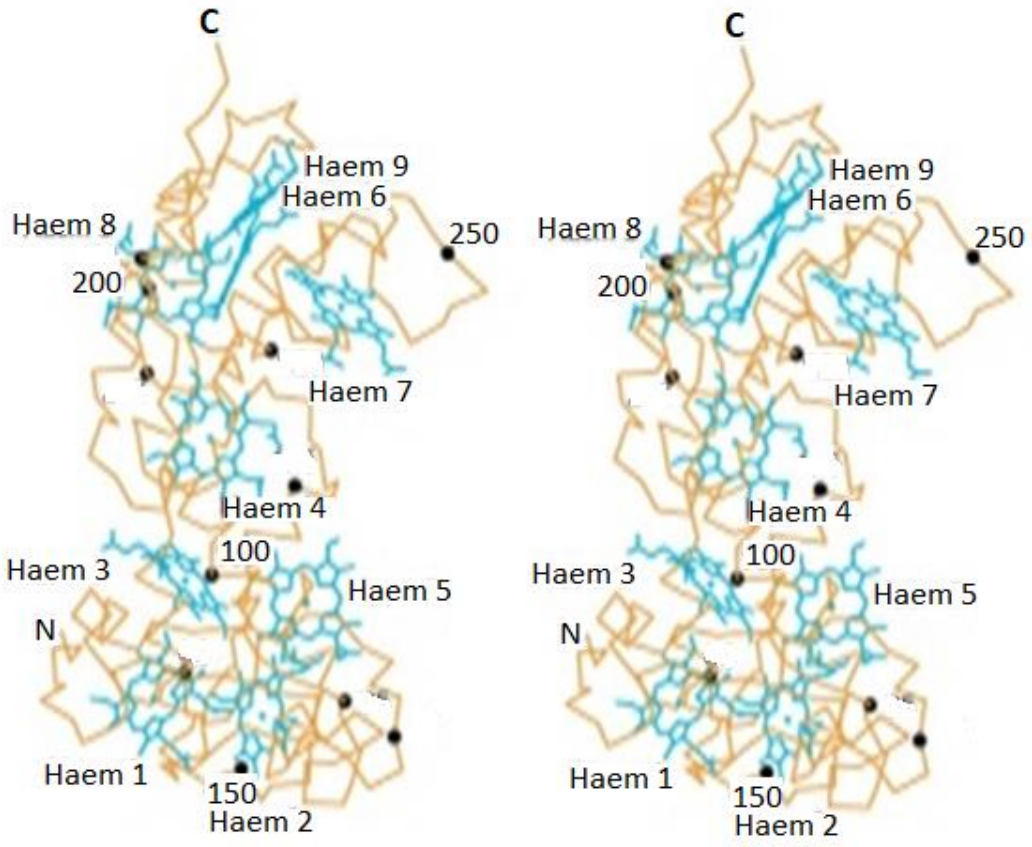


Illustration 19: High-molecular weight cytochrome C

Location of the SET-AG and SET-OP in the cells

Austin et al. suggest that Complex 1 is in the mitochondrial membrane hanging in the mitochondrial matrix (Illustration 11).

SET-AG is supposed to be located in the peroxisomes or near the cytoplasmic membrane, while SET-OP is in the intermembrane space in the mitochondrial matrix.

The Efficiency of the Multipart Electron Transfer Chain

After energy investment, energy is produced in the SET. In addition, new ATP molecules are created, and the membrane potential will be realized. At the end of the process, in addition to the newly synthetised four ATP, the ADP molecules formed during the energy investment and Nine further ADP are transformed back into ATP, using the energy and Pi produced by the transformation. The theoretical yield of the energy transformation is summarised in Table I.

	ATP [a]	A A [b]	Source molecules	Product of METC					
				O^{2-}	CO_2 + energy	H^+		Pi	H_2O
I Connecting Unit 1: one 3Fe-4S (cys-S)$_3$	1	1	3 H_2PO_4 1/2 Pyruvate	3	1,5	6		3	
II Pi-PU: four 2Fe-2S (cys-S)$_4$	4		16 H_2PO_4 4 acetic acids	16	8	32		16	
III ADP-PU: four 8Fe-7S (cys-S)$_6$	12 (4x3)	2		24 (4x6)					
A. 4UA + 4 NH_3= 4 NHUA + 4 O^{2-} B. 4 NHUA + 12 H_2PO_4 + 8 D-glucose = 4 ADP + 12 PO_3^{3-} + 4 ribose + 8 CO_2 + 24 H^+			4 NH_3 4 UA 4 NHUA 12 H_2PO_4 8 Glucose 1/3 Pyruvate	←4 8 12 →	2 8	24	4 NHUA 4 ribose = 4 Pyruvate + 4 acetic acids 4 ADP	12	4
IV Connecting Unit 2: One 4Fe-4S (cys-S)$_4$	2		4 H_2PO_4 1/3 Pyruvate	4	2	8		4	
Product of I, II, III, and IV	19			47	21.5	70		35	
V ATP synthase									
4 ADP + 4 PO_4^{3-} = 4 ATP							4 ATP		

[a] Activating molecule: ATP;
[b] Initiating molecule: Ascorbic Acid, AA;
METC: Multipart Electron Transfer Chain; Pi:PO_3^{3-}.

Table I: the hypothetical yield of energy transformation in one Multipart Electron Transfer Chain

Activation by ATP results Phosphorylation of glucose, while ADP will be formed from ATP. Nineteen ATP is responsible for the activation of the METC, resulting in 19 ADP, and seven G6P.

In each METC, 35 Pi are produced. eight are responsible for the ADP-ATP transformation of the activating ATP molecules. The remaining sixteen Pis might transform sixteen additional ADPs to ATP. Table II presents the activating and initiating molecules of the Fe-S clusters.

The hypothetical structures responsible for the energy and energy-carrier transformation must be much more complicated than

described here. Their proper functions must depend on further factors, such as the transport and stabilizing proteins, enzymes, and enzyme cofactors.

It can be assumed that several parts of the hypothesis are inaccurate and need correction. Nevertheless, the hypothesis can contribute to a better understanding of the energy supply of cells through its original approach

		Molecules of the permanent structure	Activating molecule			Initiating molecule
			ATP	Glucose	Glucose 6 phosphate	Ascorbic acid
CU1		3Fe-4S (cys-S)$_3$	1	1 →	1	1
Pi-PU		4 X 2Fe-2S (cys-S)$_4$	4	4 →	4	0
CU2		4Fe-4S (cys-S)$_4$	2	2 →	2	0
ADP-PU		4 8Fe-7S (cys-S)$_6$ 1 Flavin 1 Nicotinamide	12	8 →	4 ATP + 4 ribose	2
6 METC			19	6 x 15	6 x 7 G6P 24 ATP 24 ribose	3

Table II: Activating and initiating molecules of the Fe-S clusters

The relation of SET's hypothesis to the vitamin C-based cancer therapy

In vitro obtained results and murine experiments consequently prove the cytotoxic effect of AA on cancer cells. However, current clinical evidence for the therapeutic effect of high-dose intravenous (iv.) vitamin C therapy (HAAT) is ambiguous. The difference might be caused by the missing knowledge of AA's actions. The hypothesis described above helps to understand the iv. vitamin C's way of action. In the literature, there are many publications regarding vitamin C and cancer. Based on four review articles and the Cancer Information Summary of the National Cancer Institute's results, 20 publications were analysed related to HAAT. The results indicate that HAAT might be a useful cancer-treating tool in certain circumstances [25].

Because aerobic glycolysis produces significantly less energy, cancer cells can only be viable using more sugar. Thus, tumor cells use 200 times more glucose than healthy cells [26]. In addition, malignant tumor cells perform glycolysis ten times faster than their healthy tissue counterparts [27]. While rapidly growing tumor cells do not have adequate vessels during their genesis, the limited capillary support often results in hypoxia within the tumor. In addition, some tumor cells overexpress specific glycolytic enzymes, resulting in higher glycolysis rates, referred to as the Warburg effect [28]. The most common cellular metabolism changes involve intracellular glucose utilization and regulation loss between glycolytic metabolism and respiration [29]. Thus, tumor cells adapted to the hypoxic environment by the HIF-1α have unique energy production, realized by the low-efficiency aerobic glycolysis.

Korth et al. supposed that two L-vitamin C molecules are in the NADPH pocket, presumably near the adenine binding site in the inner membrane of the mitochondria [30]. Their conclusion is based on molecular mechanistic docking computations.

The concept regarding energy transformation describes the strong relationship between vitamin C and energy transformation. We suppose that carbamide–carboxyl of the Cys-S chains connects Complex 1, Complex 2, and Complex 3, while the CU1 and CU2 create a functional Unit from the six METCs. Vitamin C molecules are needed to initiate METCs. The condition of energy

transformation provides a continuous electron flow, warrantied by the continuity of the electron chain. In SET structures, vitamin C ensures continuity at several points. The complex, synchronized energy transformation process begins when the last connection is realized. Furthermore, suppose the energy transformation process starts, and the glucose for the reaction is unavailable there. In that case, the produced O2- radicals will kill the cells after the exhaustion of the caspase defense mechanism. A successful Vitamin C cancer therapy might be developed based on this knowledge.

Connection of the hypothesis with known facts.

The hypothesis was created based on scientific publications (Table III).

Facts, published background	Hypothesis
An ancient cell formed symbiosis with another cell, now known as the mitochondrion, and formed the eukaryotic cell [3].	Thus, eukaryotes must have two energy-transformation structures (SET-AG and SET-OP).
Knowledge regarding Fe-S clusters, iron, sulphur, and oxygen. Carosio et al published that Sodium Ascorbate induces apoptosis in neuroblastoma cell lines by interfering with iron uptake [31].	ADP-PU, METC, Illustration 3 O^{2-} produced by Fe-S clusters kill tumor cells if glucose is not available.
The possibility of carbamide–carboxy bound	R-C-NH_2 + carboxyl-R = R-C-N-C-R + H_2O; Illustration 10
Mitochondrial ETC, Illustration 11	METC; Illustration 14,; CU, ADP-PU, Pí-PU, G6P-PU
Korth et al. supposed that two L-vitamin C molecules are in the NADPH pocket of the mitochondria [30].	ATP activates, while AA initiates the Fe-S clusters.
Kinga Linowiecka et al. stated that ascorbic acid (AA) is an oxidative stress sensor and a gene expression regulator [18].	Illustrations 13, and 14.

Table III: Scientific background of the hypothesis

Conflicting Interests

The author declared no potential conflicts of interest concerning the publication of this article.

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