



Exploring the ATP Synthesis in Unique Cellular Structures: A Preliminary Hypothesis

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Abstract

Background: Adenosine Triphosphate (ATP) serves a pivotal role in cellular energetics, traditionally understood to be synthesized from Adenosine Diphosphate (ADP) and inorganic phosphate by ATP synthase. This manuscript introduces a novel hypothesis suggesting an alternative synthesis mechanism involving specific cellular structures - Structure for Energy Transformation (SET).

Objective: To outline and explore the new hypothesis that ATP synthesis occurs through a complex process within the SET, which implicates multiple chemical constituents in a distinct stoichiometry, producing two kinds of ATP, PO_3^{3-} , (Pi), and CO_2 . New ATP and ADP+Pi are formed according to the hypothesis.

Methods: The proposed experimental approach involves culturing HeLa cells in the presence of ^{18}O -labeled phosphate and assessing ATP and CO_2 contents using mass spectrography and LC-MS/MS for adenine nucleotide quantification.

Hypothesis: The SET, comprising six multiplex electron transfer chains, potentially facilitates a chemical process involving D-glucose, uric acid, NH_3 , and H_2PO_4^- molecules, leading to the synthesis of ATP and other products.

The presence of ATP containing three ^{18}O containing Pi and the characteristic of ^{18}O containing CO_2 would support the hypothesis.

Conclusion: This manuscript elucidates a preliminary hypothesis, aiming to ignite discourse and collaborative efforts within the scientific community to explore and validate this proposed mechanism of ATP synthesis in further research endeavors.

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1. Introduction

It is well known that Adenosine Triphosphate (ATP) is formed by ATP synthetase. The ATP is formed from Adenosine Diphosphate (ADP) and PO_3^{3-} (Pi). [1][2][3]

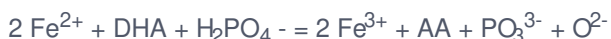
Fe-S clusters, the Fe^{3+} - Fe^{2+} change.

Fe-S clusters are the essential structures of the electron transfer chain. The central part of all Fe-S molecules is 2Fe-2S. The Fe atom may have three or two electrons. Both Fe atoms bind two cys-S components ($\text{HOOC}-\text{CH}(-\text{NH}_2)-\text{CH}_2-\text{S}-\text{R}$). [4][5]

Sulphur – Oxygen change

The affinity of Fe^{3+} to OH is more extensive than to S. Binding OH by Fe^{3+} results in two electrons (Fe^{2+}) as H^+ will leave the molecule, creating the membrane potential. Later in the Fe-S cluster, Fe^{2+} will become Fe^{3+} by the hydrogen atoms of the H_2PO_4^- molecules. [6]

Ascorbic acid (AA) and ATP can change the S to OH in the Fe-S clusters. First, ATP activates the Fe-S clusters by two OH, resulting in energy transfer. After this, the cluster is ready for function. [7]



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 co-occurring) and couples this electron transfer with the transfer of protons (H^+ ions) across a membrane. In the mitochondrion, four 2Fe-2S, one 3Fe-4S, and seven 4Fe-4S clusters offer the proper function of Complex I, Complex II, and Complex III, as described by Austin et al. [8]

2. Hypothesis Explanation

Glucose and ATP are the most essential energy carriers of the cells. During the energy liberation of glucose, ATP might be formed in specific structures of the cells. Eukaryote cells have the Structure for Energy Transformation of Anaerobic Glycolysis (SET-AG) and the SET of Oxidative Phosphorylation (SET-OP). Accordingly, eukaryote cells can survive in hypoxic–anoxic conditions. [9]

The Fe-S clusters are supposed to be connected by cys-S bounds, forming one Multiplex Electron Transport Chain (METC), where the transformation is completed. [9] Four 2Fe-2S clusters might be responsible for Pi production, forming a Pi-PU. The 3Fe-4S cluster might form the Connecting Unit 1 (CU1). Instead of the seven 4Fe-4S clusters suggested by Austin et al. [8], four 8Fe-7S clusters of nitrogenase -forming the Adenosine Triphosphate Producing Unit (ADP-PU) - and one 4Fe-4S cluster – the Connecting Unit 2 (CU2) are in the METC. Thus, CU1, ADP-PU, Pi-PU, and CU2 might create the METC. Six METCs might form the SET. All METC produce four ATP, 23 Pi (35- 3x4), 47 O²⁻, 70 H⁺ and 21,5 CO₂. The 47 O²⁻ must result in H₂O or react with carbon, producing CO₂. The carbon atom of the CO₂ originates from glucose, acetic acid and Pyruvate molecules. [7] ATP synthetase and many other specific enzymes are responsible for the proper function of the METC. The reaction of the SET starts when all segments of the chain are connected. AA plays a determinant role in the realization of continuity.

The ATP-Producing Unit (ATP-PU)

Eight D-glucose, four uric acid (UA), four aminated UA (NH-UA) and twelve H₂PO₄⁻ molecules are bound to four 8Fe-7S clusters. In addition, four NH₃ molecules are also needed for the amination of the UA molecules (Illustration 1).

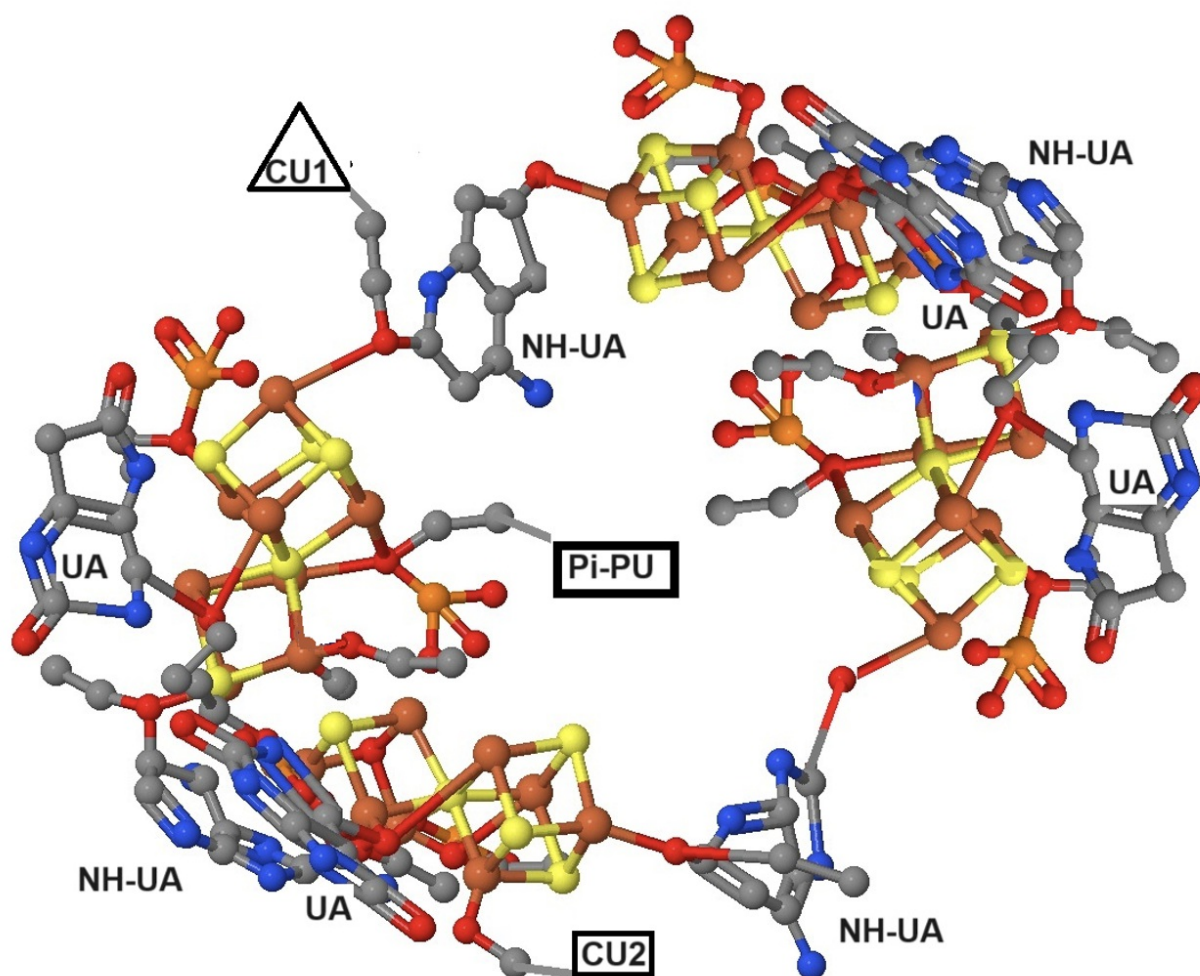


Illustration 1. The ADP-producing unit of the multiplex electron chain. Abbreviations: CU1: Connecting Unit 1; NH-UA: aminated uric acid; UA: uric acid; Pi-PU: PO_3^{3-} Producing Unit; CU2: Connecting Unit 2. The eight D-glucose and the four NH_3 molecules are not illustrated.

The SET-AG comprises six METCs, while the SET-OP has six METCs + two high molecular cytochromes.

H_2PO_4^- originated Pi, O^{2-} , CO_2 and H^+ Products of the METCs

The CU1, ADP-PU, Pi-PU, and CU2 units produce Pi, O^{2-} and H^+ from H_2PO_4^- . CU1 produces three, ADP-PU twelve, Pi-PU $4 \times 4 =$ sixteen, while CU2 four Pi (Table I).

Table I. Pi, O^{2-} , H^+ and ATP products of the METC

	Pi	O ²⁻	H ⁺	ATP
CU1 – 3Fe-4S	3	3	6	
ADP-PU 4 8Fe-7S	12	12	24	4
PiPU 4 2Fe-2S	4x4 = 16	16	32	
CU2	4	4	8	
METC	35	35	70	
SET (6 METCs)	210	210	420	24

Abbreviations: Pi: PO₃³⁻; CU1: Connecting Unit1; ADP-PU: ADP Producing Unit; PiPU Pi-Producing Unit; CU²: Connecting2; METC: Multiplex Electron Transport Chain; SET: Structure for Energy Transformation;

According to the hypothesis, six times (eight D-glucose, four UAs, four NH₃, and 35 H₂PO₄⁻ molecules) produce 129 CO₂, 6X4 H₂O, 6X4 new ATP and 6x23 Pi. The twelve Pi produced in the ADP-PU will form ATP, while the rest (twenty-three) will form ATP from ADP by the ATP synthetase (Table II). [7][9]

		O ²⁻	CO ₂	Pi	H ⁺	NHUA	R5P	ribose	ATP
METC		47	21,5	35	70	4	8	4	4
CU1	3Fe-4S (cys-S)	3	1,5	3	6				
Pi-PU	4 2Fe-2S (cys-S)	4x4 16	8	16	32				
ADP-PU	4 8Fe-7S (cys-S) 6 1 Flavin 1 Nicotinamide	24	12	12	24				
CU2	4Fe-4S (cys-S)	4	2	4	8				
SET	6 METCs	282	129	210	420	24	48	24	24

Table II. The hypothetical products of METC's Units Abbreviations: METC: Multiplex Electron Transport Chain; SET: Structure for Energy Transformation; R5P: Ribose 5 phosphate.

In vitro experiments using ¹⁸Oxygen labelled H₂PO₄⁻ molecules might underline the hypothesis.

3. Material and methods (*must be corrected by the used techniques*)

HeLa cells should be cultured in the presence of NaH₂P¹⁸O₄. Cells of four-day cultures must be investigated by mass spectrograph regarding the ATP and CO₂ content. Adenine nucleotide extraction and quantification by LC-MS/MS. ATP, ADP, and AMP should be extracted from cells, processed and quantified by liquid chromatography with tandem mass spectrometry detection (LC-MS/MS), as described by Richani D et al. [10][11]

Gas chromatography should be used for analysis of the CO₂.

4. Expected Outcomes

ATP

According to the hypothesis, the SET consists of six METCs, each producing four ATP and twenty-three Pi (Table I). Accordingly, the mass spectrum investigation will result in three kinds of ATP molecules. Thus, in addition to the unlabeled ATP molecules (A), two further molecules (B and C) must be detected (Table III and Table IV).

Table III. Number of labelled molecules in ADP and ATP

		Unlabeled	Labeled
A	ATP	3	0
B	New ATP (ADP + Pi)	2	1
C	New ATP (NHUA+ 3 Pi)	0	3

Because of the ¹⁸O content of Pi, they will result in 3x2=6 neutron excess in the B (ADP+ labelled Pi), while 3X6=18 excess in the C (newly produced ATP) molecules. The predicted ratio of B to C is 23 to 4 =5,75 (Table I).

ADP

The molar mass of ADP is 427,2.

Table IV. ATP monitored transitions (m/z) and collision energy used for stable isotope tracing.

	Nucleotide	Neutron excess	(m/z)	Collision Energy (V)
A	ATP		508.2>136.2	25
B	1 X P ¹⁸ O ₃ -ATP	6	514.0>136.2	25
C	3 X P ¹⁸ O ₃ -ATP	18	526.0>136.2	25
D	ADP		427,2	25

CO₂

The labelled H₂PO₄⁻ and the unlabeled UA molecules give the oxygen content of CO₂. Thus, if the cells are cultured in anoxic condition, one part of the CO₂ will contain one labeled oxygen (B; one coming from NHUA and one coming from

Pi), while others will contain two labeled oxygens (C; both coming from Pi). Accordingly, the mass spectrograms must contain three peaks. The unlabeled CO₂ (44.0) (A), the B (+ 2 neutrons). C (+4 neutrons). The predicted ratio of C to B is 8 to 39 = 4,875 (Table V).

CO₂ analyzed with a gas chromatograph must be tested for ¹⁸O content.

Table V. CO₂ monitored transitions (m/z) and collision energy used for stable isotope tracing.

	Nucleotide	Neutron excess	(m/z)	Collision Energy (V)
A	CO ₂		40.0>136.2	25
B	CO ¹⁸ O	2	42>136.2	25
C	C ¹⁸ O ₂	4	44>136.2	25

5. Discussion

Potential implications if the hypothesis were to be validated by subsequent research

The hypothesis predicts that AA initiates the energy transformation, where ATP, CO₂, H⁺, acetic acid Pyruvate molecules and energy are produced from D-glucose molecules. It is well known that Adenosine Triphosphate (ATP) is formed by ATP synthetase. The ATP is formed from Adenosine Diphosphate (ADP) and PO₃³⁻ (Pi). ^{[1][2][3]} Accordingly, detection of ATP containing three labelled Pi might support the hypothesis.

The ratio of 4.875 regarding one or two ¹⁸O containing CO₂ molecules would offer further confirmatory data.

Potential limitations and challenges that may arise during experimental validations

- One part of the newly synthesized ATP participates in the cell metabolism, influencing the predicted ratio of the new ATP (Table III C) to the ADP- ATP transformed (Table III B) molecules (5,75).
- It is possible that the amount of the ADP- ATP transformed (B) molecules will significantly exceed the assumed amount. In that case, the ADP- ATP transformation can also be created outside the SET.

6. Collaboration Invitation

From the reader, feedback and insights are called.

We are looking for a collaborating partner.

Further manuscript versions will be developed and updated based on collective feedback.

7. Conclusion

This manuscript elucidates a preliminary hypothesis, aiming to ignite discourse and collaborative efforts within the scientific community to explore and validate this proposed mechanism of ATP synthesis in further research endeavors.

The results of the experiments can support or reject the assumptions of the hypothesis. They can also answer whether there are other ADP-ATP transforming structures than the SETs. The hypothesis opens the way to a successful intravenous vitamin C treatment of cancer.

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