# 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 which proposes that ATP synthesis occurs through a complex process within the SET, which implicates multiple chemical constituents in a distinct stoichiometry, resulting in the production of ATP, $PO_3^{3-}$, (Pi), and $CO_2$.

**Methods:** The proposed experimental approach involves culturing HeLa cells in the presence of $^{18}$Oxygen-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.

**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 ($HOOC–CH(–NH_2)–CH_2–S-R$). [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]

$2\ Fe^{3+} + AA = 2\ Fe^{2+} + DHA + H^+$

$2\ Fe^{2+} + DHA + H_2PO_4^- = 2\ Fe^{3+} + AA + PO_3^{3-} + O^{2-}$

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 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, 47 $O^{2-}$, 70 $H^{+}$ and 21,5 $CO_2$. The 47 $O^{2-}$ must result in $H_2O$ or react with carbon, producing $CO_2$. The carbon atom of the $CO_2$ 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 $H_2PO_4^{-}$ molecules are bound to four 8Fe-7S clusters. In addition, four $NH_3$ molecules are also needed for the amination of the UA molecules (Illustration 1).

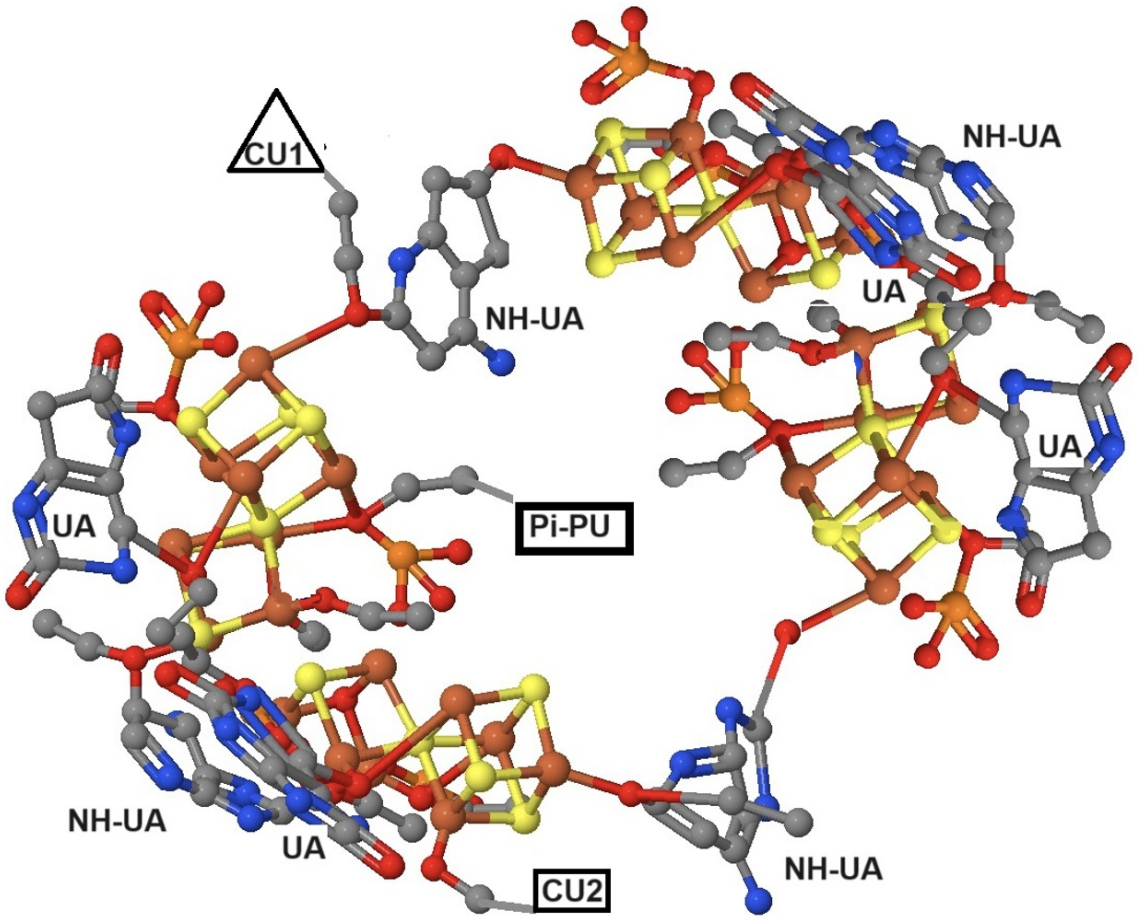


**Illustration 1.** The ADP-producing unit of the multiplex electron chain.

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 NH3 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 4x4 =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 | |
| PiPU 4 2Fe-2S | 4x4 = 16 | 16 | 32 | 4 |
| CU2 | 4 | 4 | 8 | |
| METC | 35 | 35 | 70 | |
| SET (6 METCs) | 210 | 210 | 420 | 24 |

According to the hypothesis, six times (eight D-glucose, four UAs, four $NH_3$, and 35 $H_2PO_4^-$ molecules) produce 129 $CO_2$, 6X4 $H_2O$, 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]

In vitro experiments using $^{18}$Oxygen labelled $H_2PO_4^-$ molecules might underline the hypothesis.

| | | $O^{2-}$ | $CO_2$ | Pi | $H^+$ | NHUA | G5P | ribose | ADP |
|---|---|---|---|---|---|---|---|---|---|
| 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<br>1 Flavin<br>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

Abbreviation: G5P: glucose 5 phosphate

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

HeLa cells should be cultured in the presence of $NaH_2P^{18}O_4$. Cells of four-day cultures must be investigated after deep freeze by mass spectrograph regarding the ATP and $CO_2$ 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 $CO_2$.

## 4. Expected Outcomes

### ATP

According to the hypothesis, the SET consists of 6 Multiplex electron transfer chains, each producing four ATP and twenty-three Pi. 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).

Because of the $^{18}O$ content of Pi, they will result in 3x2=6 neutron excess in the B, while 3X6=18 excess in the C molecules. The predicted ratio of B to C is 23 to 4 =5,75 (Table III).

### ADP

The molar mass of ADP is 427,2. The new ATP originated, labelled $^{18}O$ containing ADP, must have a 439,2-molar mass.

**Table III.** 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 23** | 1 X $P^{18}O_3$-ATP | 6 | 514.0>136.2 | 25 |
| **C 4** | 3 X $P^{18}O_3$-ATP | 18 | 526.0>136.2 | 25 |
| **D** | ADP | | 427,2 | 25 |
| **E** | 2 X $P^{18}O_3$-ADP | 12 | 439,2 | 25 |

### $CO_2$

The labelled $H_2PO_4^-$ and the unlabeled UA molecules give the oxygen content of $CO_2$. Thus, one part of the $CO_2$ will contain one labeled Oxygen (B), while another will contain two labeled Oxygens (C). Accordingly, the mass spectrograms must contain three peaks. The unlabeled $CO_2$ (44.0) (A), the B (+ 2 neutrons). C (+4 neutrons) and the predicted ratio of C to B is 81 to 48 = 1,68 (Table IV).

$CO_2$ analyzed with a gas chromatograph must be tested for $^{18}O$ content.

**Table IV.** $CO_2$ 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 limitations and challenges that may arise during experimental validations.

a. 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).
b. 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.

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

The hypothesis predicts that AA initiates the energy transformation, where ATP, $CO_2$, $H^+$, acetic acid Pyruvate molecules and energy are produced from D-glucose molecules. The process of transformation results in all METC producing 47 $O^{2-}$; they might destroy the cell if the glucose molecules are not available in the cell. Successful treatment of cancer diseases might be developed based on this knowledge,

## 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.

## References


1. ^Nakamoto, R. K., Baylis Scanlon, J. A., & Al-Shawi, M. K. (2008). The rotary mechanism of the ATP synthase. Archives of Biochemistry and Biophysics, 476(1), 43–50.
2. ^Hahn, A., Vonck, J., Mills, D. J., Meier, T., & Kühlbrandt, W. (2018). Structure, mechanism, and regulation of the chloroplast ATP synthase. Science, 360(6389), 4318. https://doi.org/10.1126/science.aat4318
3. ^Morales-Rios, E., Montgomery, M. G., Leslie, A. G. W., & Walker, J. E. (2015). Structure of ATP synthase from Paracoccus denitrificans determined by X-ray crystallography at 4.0 Å resolution. Proceedings of the National Academy of Sciences of the United States of America, 112(43), 13231–13236. https://www.pnas.org/content/112/43/13231
4. ^Sanchez, M., Sabio, L., Galvez, N., Capdevila, M., & Dominguez-Vera, J. M. (2017). Iron Chemistry at the Service of Life. IUBMB Life, 69, 382–388.
5. ^Johnson, D. C., Dean, D. R., Smith, A. D., & Johnson, M. K. (2005). Structure, function, and formation of biological iron-sulfur clusters. Annual Review of Biochemistry, 74, 247–281.
6. ^Linowiecka, K., Foksinski, M., & Brożyna, A. A. (2020). Vitamin C Transporters and Their Implications in Carcinogenesis. Nutrients, 12, 3869. https://doi.org/10.3390/nu12123869
7. a, b, cHunyady, J. (2023). Cell energetics and ascorbic acid. Cancer treatment with vitamin C. Submitted to International Journal of Molecular Sciences.
8. a, bAustin, D. R., Rachel, E. T. B., Stephen, L. A., & Kimberly, J. D. S. (2021). Mitochondrial iron–sulfur clusters: Structure, function, and an emerging role in vascular biology. Redox Biology, 47, 102164.
9. a, b, cHunyady, J. (2023). The dual energy supply of eukaryotic cells. Earth & Environmental Science Research & Reviews, 6(2), 438-453.
10. ^Richani, D., Lavea, C. F., Kanakkaparambil, R., Riepsamen, A. H., Bertoldo, M. J., Bustamante, S., & Gilchrist, R. B. (2019). Participation of the adenosine salvage pathway and cyclic AMP modulation in oocyte energy metabolism. Scientific Reports, 9, 18395. https://doi.org/10.1038/s41598-019-54693-y
11. ^Zhang, G., Walker, A. D., Lin, Z., Han, X., Blatnik, M., Steenwyk, R. C., & Groeber, E. A. (2014). Strategies for quantitation of endogenous adenine nucleotides in human plasma using novel ion-pair hydrophilic interaction chromatography coupled with tandem. Journal of Chromatography A, 1325, 129-136. https://doi.org/10.1016/j.chroma.2013.12.017