Review of: "Why We Stop Synthesizing Essential Amino Acids: The Extracellular Protein Hypothesis"

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Potential competing interests: No potential competing interests to declare.

The work proposes an interesting hypothesis to explain the occurrence of essential amino acids in living organisms. The hypothesis is based on 2 ideas: i) intracellular proteins are the main source of amino acids for protein synthesis and ii) extracellular proteins should have fewer essential amino acids than intracellular ones.

While the idea is appealing, there are serious flaws in this work. I'll describe these in the next lines to help the author improve this work.

Main concerns

1. The author presented data (Figure 1) that correlates hydrophobicity and synthesis cost of each amino acid. In such representation, the essential amino acids appear at the top of the graph. The author argues that the boundary separating essential from non-essential amino acids “does not align perfectly”. Then the author presents Figure 2, where the author argues there is a boundary between essential and non-essential amino acids based on PCA that indicates an “underlying, yet unknown, correlation”. Finally, in Table 3, the authors present composition data of extracellular and intracellular proteins in the transition from egg to chicken and argue that “a disparity in amino acid composition exists between intracellular and extracellular compartments”. Thus, three pieces of evidence are presented: one supporting the notion of synthesis cost as the main force behind the essentiality of amino acids, and the other two relating amino acid composition in food or cellular compartments. Which one has stronger support? The authors argue that the last two pieces of evidence are consistent with the hypothesis, but that does not establish its validity. The author needs to provide a way to measure, in a quantitative way, how well the data separates essential from non-essential amino acids. Based on that analysis, the authors may have better arguments to support the hypothesis.
2. It is important for the author to make a more extensive review of the literature, as noted above. For instance, bacteria also produce a large number of extracellular proteins, but they indeed vary with habitat structure and community diversity. See reference Garcia-Garcera, M., Rocha, E.P.C., Community diversity and habitat structure shape the repertoire of extracellular proteins in bacteria, Nat Commun 11, 758 (2020). https://doi.org/10.1038/s41467-020-14572-x. In such a reference, the authors compared the cost of synthesis of intracellular and extracellular proteins and cited

previous works that have proposed that extracellular proteins include "less expensive" amino acids than intracellular proteins. In this work, the author claims this is an original idea of this work and only presented the data for the egg-chicken transition to support this claim. The cited work published in Nat. Communications includes a variable, habitat of bacteria, and found that the cost of synthesizing extra- and intra-cellular proteins is directly related to the habitat.

3. References 3-6 are constantly cited throughout the text, suggesting the ideas presented in those works are the main support of this hypothesis, but also that the author has not reviewed other literature that may provide alternative explanations for the essentiality of amino acids. For instance, if the author had reviewed the proportion of auxotrophs and heterotrophs in the biosphere, it would have been evident that the majority and most abundant species seem to be auxotrophs, hence the strategy to eat others to acquire essential amino acids seems odd based on this context. Furthermore, while it is true that many animals share similar essential amino acids, the idea that such is the consequence of developing a feeding ability seems not strong enough. For instance, some bacteria cannot produce several amino acids (see, for instance, Lactobacillus plantarum: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7345125/, or others reported elsewhere: https://www.nature.com/articles/s41598-020-69686-5). One may wonder if for all the bacteria reported to require some essential amino acids, these would live in niches where the essential amino acids are freely available (?) or they develop a way to kill other living forms like animals, as the authors proposes; in the previous point, it is argued that the habitat matters. Another area that was not revised by the author is autophagy. Autophagy is downregulated by an excess of free amino acids within cells; particularly, Leu is known to effectively inhibit autophagy. Autophagy is activated when part of the cell is not working properly, so such parts are degraded in lysosomes and recycled. How such an autophagy cycle would fit into this hypothesis?

Minor concerns

1. The authors did not cite several of the references correctly. For instance, cite 22 is referred to by the author using data that seem to reflect young adults, while the author reports this for humans. References 2-6 are used in the Introduction, which does not support the text citing them (e.g., “The historical and simplest explanation for this phenomenon is that humans did not need to synthesize these amino acids due to their abundance in their diet (3-6),” or “This suggests an origin at the level of a common ancestor shared by humans and these animals, with this trait being inherited by their descendants (2-6)”).
2. Reference 7 includes a DOI that is not found.
3. The following phrase is misleading: “This primarily stems from the fact that autotrophic organisms, such as plants and fungi, which lack the capability to ingest, do not require amino acids.” Plants and fungi do need amino acids.
4. The data to build Figure 2 needs to be provided and explained as to how these data were generated.
5. The title “Can the Amino Acid Composition Disparity Between Intracellular and Extracellular Explain Essential Amino Acids?: A Hypothesis” needs rephrasing.
6. There are many other sentences in this document that mislead the reader to ideas that are not precise. I do not provide a complete list, just some examples. In addition to those listed above, another example would be: "If so, the classification of arginine as essential may not stem from a lack of synthetic capability but rather from the increased

demand within the urea cycle for processing ammonia, a byproduct of extensive protein degradation during events such as starvation or development." The authors claim Arg should be considered essential because of the urea cycle, a cellular process not found in all animals. The author needs to review carefully the statements presented in this document.