Phylogenetic Analysis of AP Complexes

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Abstract

Eukaryotic organisms possess compartmentalized, membrane-bound organelles. To transport cargo between and into these compartments, there must be a highly regulated system of proteins that control vesicle formation and movement. Adaptor proteins (APs) function to mediate endocytosis by preparing a clathrin-coated vesicle, which is essential for interaction with the environment and is commonly manipulated by viruses. Many organisms across the eukaryotic phyla share various types of APs. This project analyzes the evolution of AP-1 and AP-2 by constructing phylogenetic trees of each of the four subunits across one hundred different species. Similar research has been done on this subject; however, such work had often studied a larger range of proteins over a smaller range of species. This study will help elucidate the specific conserved genes and mechanisms between species as they evolved over time. These sequences were downloaded and processed through a phylogenetic software program created by the National Institutes of Health. The results are related to similar onsets of AP subunits between species, reflecting a similar common ancestor. By understanding the evolution of APs 1 and 2, and their associated assembly proteins, similar methods could be applied to other members of the AP family, including those which have just been discovered. This leaves a promising future for AP discovery and comparison, as well as further insight into the methodology of uncovering mechanisms of cell survival and disease prevention.

Background

AP1 regulates cargo traveling from the Golgi apparatus to endosomes and endosomes to other areas. AP2 regulates cargo traveling from the plasma membrane to endosomes. Because APs regulate trafficking, they are frequently targeted and hijacked by viruses to negatively affect immunity.

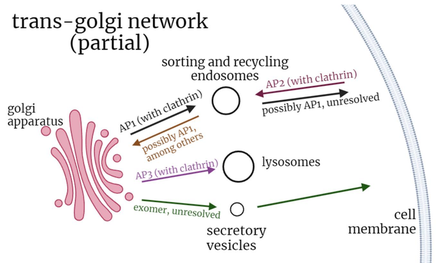

Figure 1: visual representation of some of the proteins and organelles that manage vesicular transport. The specific pathways of cargo are largely unknown and regularly a subject of debate.

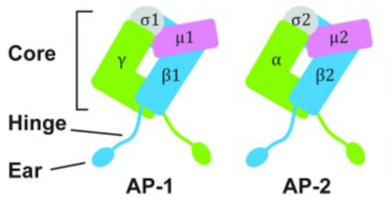

Figure 2: Simple diagrams of the subunits of AP1 and AP2. Both are heterotetrameric complexes. They are quite similar structurally but, interestingly, perform different functions.

Methodology

- We will be using UniProt, a protein sequence and function database, to search for protein sequences of each subunit .
- In addition to UniProt, we will also be searching for protein sequences on NIH.
- Then we will be aligning and comparing sequences by species with software .
- Using BLAST, an algorithm/software, we will create our phylogenetic trees.

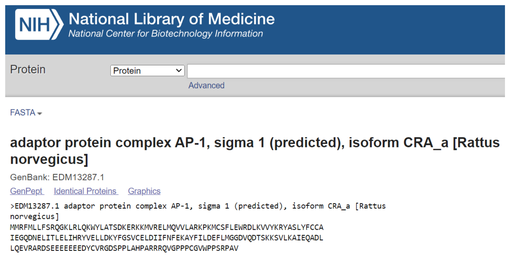

Figure 3: example of a protein sequence in FASTA formatting. Each organism subunit has a different sequence which will be aligned.

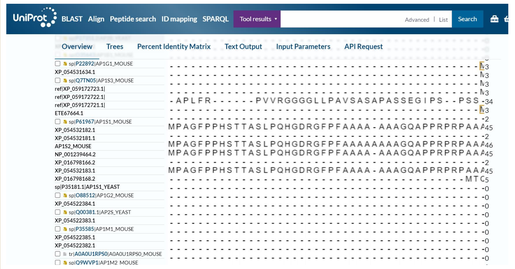

Figure 4: results of aligning sequences through UniProt. This collection of aligned sequences will be used to create a phylogenetic tree.

Preliminary Results

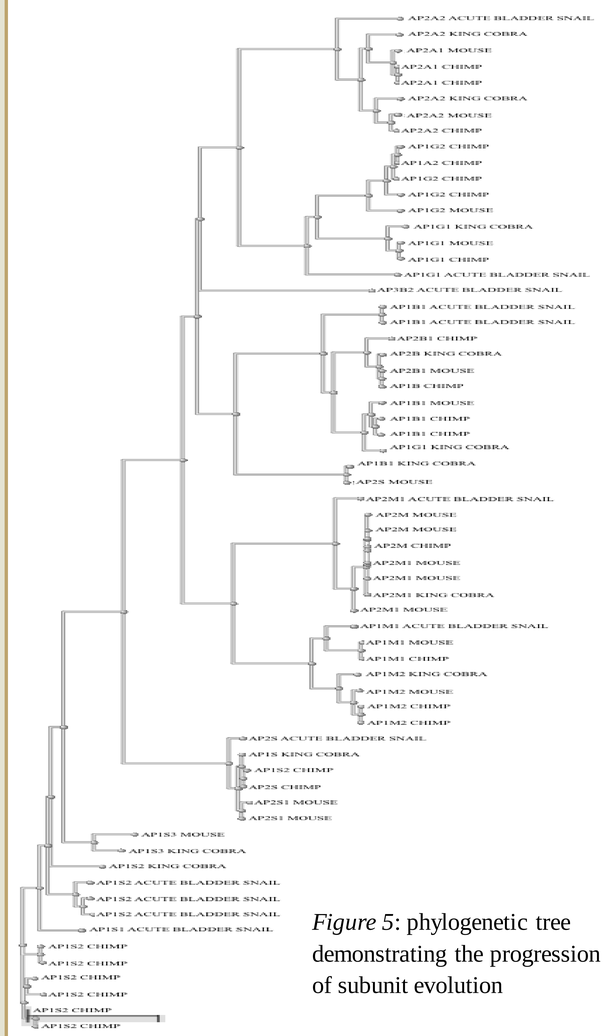

Figure 5: phylogenetic tree demonstrating the progression of subunit evolution

Implications and Future Plans

In the future, we hope to construct a tree with a more extensive range of organisms. This would provide us with more accurate results. In addition, we might be able to provide broader context with which to examine the evolutionary development of AP subunits. However, we are still looking for the most efficient and accurate methods of aligning protein sequences and creating a phylogenetic tree. Further research could lead to the investigation of the protein that facilitates the formation of these subunits, AAGAB. AAGAB stabilizes the intermediary forms of AP1 and AP2, but not the other APs.
The results of this research could advance the knowledge of the similarities and differences in the composition of AP1 and AP2 subunits between organisms. Hopefully, we can then apply this information to further improve our understanding of immunology.

Acknowledgements

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