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Tracing evolutionary history: bringing extinct proteins back to life



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Introduction

Pentameric ligand-gated ion channels are transmembrane proteins that play an important role in signal transduction in our nervous system. Upon binding of a chemical messenger, a conformational change is induced in the protein to allow the transmission of the signal into the cell, ensuring a response.

One of the superfamily members of these ion channels are **acetylcholine receptor (AChRs)** family. This study focuses specifically on the α -4 subunit of the nicotinic AChR. The gate of the receptor is opened by the binding of ligands, which in this case are two acetylcholine neurotransmitters. When the ligands are bound, a conformational change occurs in all five subunits, opening the channel pore, and allowing ions to travel across the membrane to transduce the extracellular signal. This opening requires that all five subunits are compatible and therefore functional.

This goal of this experiment is to uncover the evolution and origin of the amino acid sequences required for subunit compatibility. The findings of this study will help understand the origins of present-day ion channel structure and function.

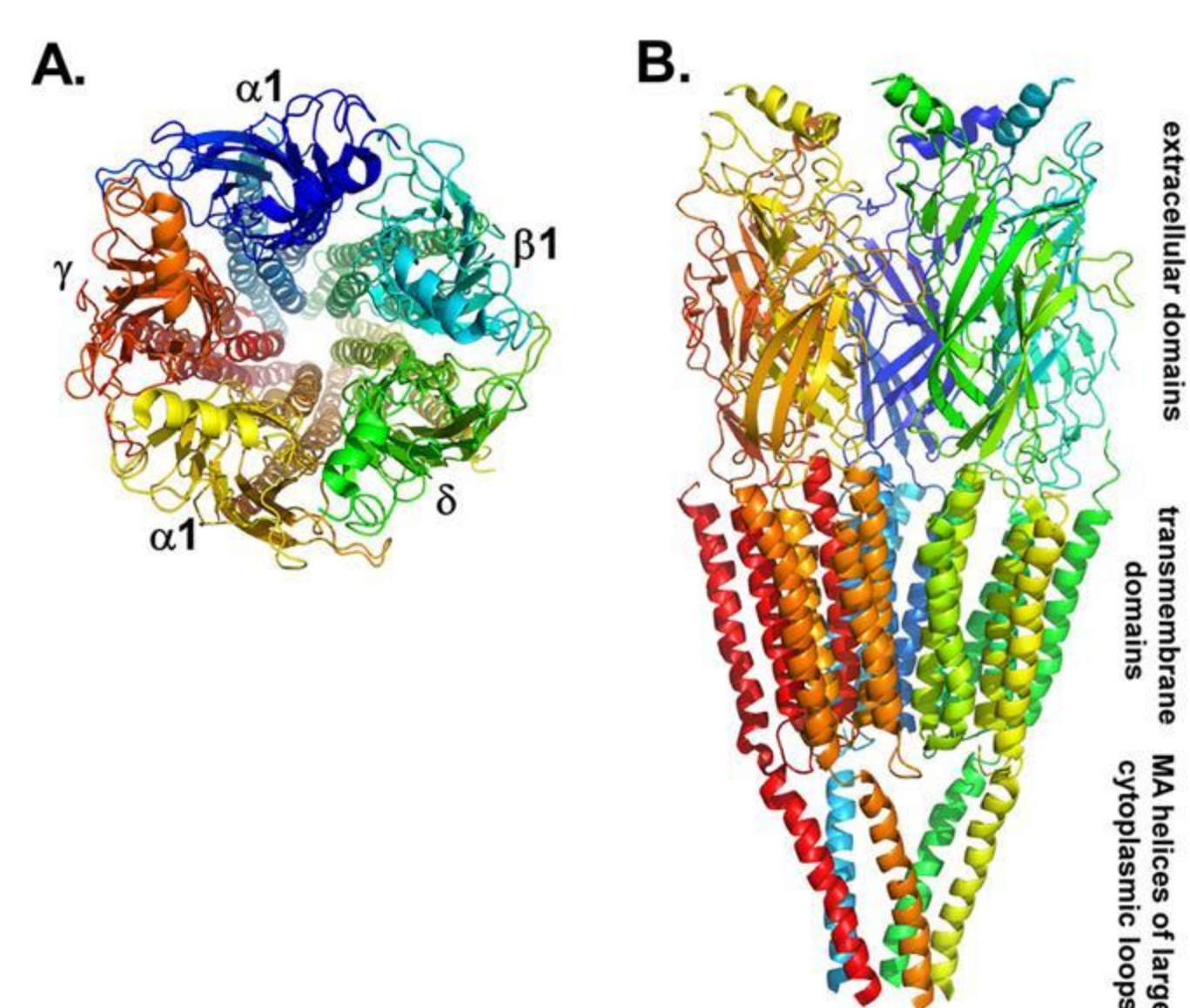


Figure 1. Top (A) and side (B) view of the AChR showing its five subunits

Methodology

- Species with similar amino acid sequences to the human sequence of the subunit were searched for in the National Centre for Biotechnology Information, or NCBI, database.
- The search resulted in 245 sequences being collected and aligned using the Jalview sequence alignment software. Sequences that are too long or too short as well as any sequence with unknown amino acid substitutions were deleted.
- The resulting alignment was used to create a phylogenetic tree. To find the ancestral α -4 subunit, a phylogenetic tree relating subunits from multiple species is created, which was refined by comparing it to the Tree of Life.
- The phylogenetic tree relates orthologous sequences to the α -4 subunit allowing us to find ancestral sequence to reconstruct.

Results

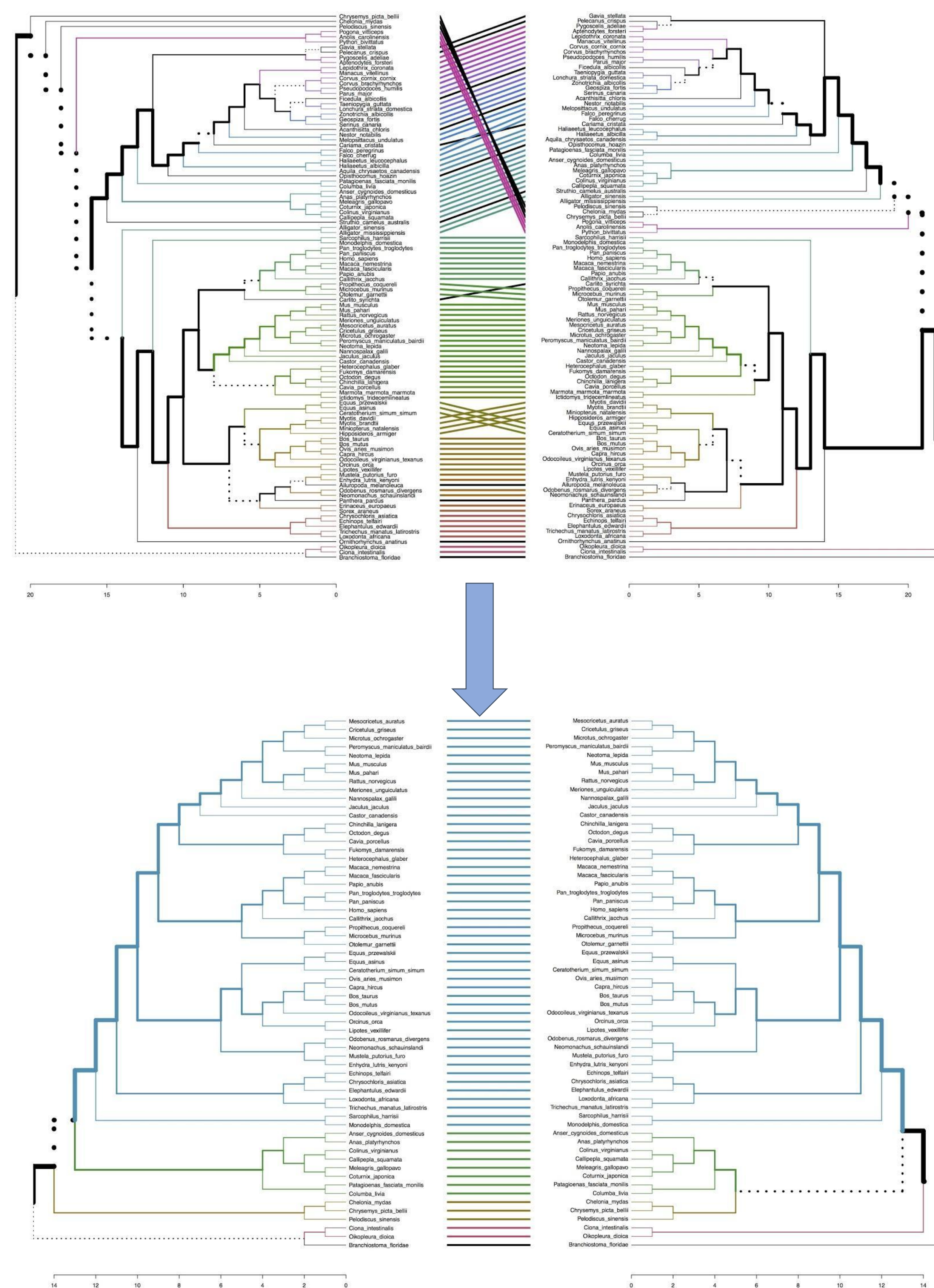


Figure 2. Tanglegrams comparing the phylogenetic tree of the sequence alignment to the Tree of Life.

Above are two tanglegrams, the top was one of the first trees produced, the bottom figure is the most recent. Several prunings of sequences were done to match the trees. It's important that we get a matching tree to ensure that the sequences are actually related in the molecular phylogeny so that we can have an accurate ancestral sequence.

The goal is to achieve a molecular phylogeny that accurately represents the evolutionary relationships of the α -4 subunit. The next steps would be to try fixing the last unmatched bits of the tree and get a clean tree to pinpoint the ancestral sequence to reconstruct.

To fix the unmatched regions of the tree different outgroups can be used. Outgroups are important for comparing the ingroup organisms and determining their evolutionary relationships, trying different outgroups could fix the phylogenetic tree and show a more clear molecular phylogeny.

Conclusion

Through an evolutionary biochemical approach, we can find an ancestral amino acid sequence of the human α -4 subunit. Once the ancestral sequence is determined, we can reconstruct this protein in the lab and test the compatibility with the other subunits of the AChR. The idea is to determine the parts of the protein that are important in subunit compatibility and therefore protein functionality.

If we can better understand how these ion channels work in our bodies, we can use this information to control the function of these ion channels, that are important drug targets, and help with advancements in medicine.

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