Evolutionary history of the chorus frog (*Pseudacris ferarium*) gamma aminobutyric (GABA) receptors

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Abstract

The chorus frog (*Pseudacris ferarium*) is a species of amphibian that uses acoustic signals for mating. Female preferences for these signals are performed through activation of neural circuits in the chorus frog central nervous system. However, very little research exists concerning the evolution of these neural circuits, specifically with respect to the evolution of neurotransmitter proteins that determine the circuit behavior. To conduct this research, I extracted transmitter proteins transcript sequences (i.e., GABA subunits) from a reference transcriptome and mapped those sequences to the chorus frog genome. I then visualized the resulting mapping in Geneious Prime to identify relative genome positions of the transcripts. The purpose was to determine if any of the transmitter genes were physically linked in the genome, in addition to basic information about the number of exons in each gene. Of the 29 transmitter genes obtained from the reference transcriptome, 31% mapped to the genome. This indicates the genome is incomplete. One interesting finding of this study is that two of the transcripts were overlapping in genomic position but in reverse orientation.

Introduction

The chorus frog (*Pseudacris ferarium*) is a species of amphibian capable of vocalization through different pulsed calls, which vary across the species range, especially when multiple species of chorus frog are present. Furthermore, divergence in female preference amongst sympatric populations also reduces the tendency to hybridize (Lemmon 2009). It is also important to note that female *P. ferarium* are more susceptible to changes in their neural networks than male chorus frogs (*Ospina et al,* 2021). One way that many chorus frogs differentiate their calls is through the number of pulses in each call. Chorus frogs are also capable of differentiating the speed of the pulses in each call through special neurons called interval counting neurons (ICNs), which count the time between different pulses, in effect measuring the speed of the call. Female response is performed through activation of signaling molecules in the synapses and neurons such as ICNs of the chorus frog brain. Spikes of activity have also been observed in ICNs and other neurons when female chorus frogs hear calls of their species (Naud *et al* 2015). By studying how closely related certain protein sequences are through analysis of positioning and the number of exons, I can gain a greater understanding of which genes underlying neural circuits may evolve in concert.

The reaction of a chorus frog to a potential mate's call is dictated by its Gamma-aminobutyric acid (GABA) protein receptors. GABA receptors are a common signal transduction pathway found in almost all animals in the central nervous system (Tsang 2007). There are two main types of GABA receptors, GABA A and GABA B. GABA A receptors are ligand gated, requiring a chemical compound to activate them. GABA A has 19 subunits, while GABA B only has two subunits. GABA A deals with the regulation of neurotransmitter proteins while GABA B mostly regulates signal transduction pathways (Olsen 2008). This project focused on GABA A receptors. Despite our knowledge of the function and roles of GABA receptors in *P. ferarium* and other organisms, very little research has been done on the positioning of GABA and protein gated ion channel gene sequences in the chorus frog genome. The similarities in the positioning of genes of different populations can indicate that certain genes have evolved together. Once we understand the genomic positions of these subunits, we can gain further understanding as to how evolution of these genes have shaped diversification of the species.

Figure 1. *Pseudacris feriarum*

Methods

I started out my research by extracting transcripts using GABA keywords that I needed to use for analyzing the RNA transcripts in Geneious Prime. I then mapped these GABA transcripts to the chorus frog genome using hisat, which is a computational technique used to compile genetic transcripts from a transcriptome. I then visualized this mapping in Geneious using the applications feature to simulate the transcription of the transcripts into RNA codons, which then can be analyzed using start and stop codons. I then computed the distance between the different regions of the transcripts that are used for coding proteins and I identified overlapping genes by using different reading frames.

References

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Results

After mapping the chorus frog gene using Geneious Prime, I observed the different scaffolds and the number of exons, or coding regions, on them. Scaffold one had eight exons, Scaffold 77 had six, Scaffold 197 had six, Scaffold 803 had one, Scaffold 919 had eight, Scaffold 4053 also had eight, Scaffold 7885 had six, while Scaffold 8989 had three. There were 29 reads found, all 29 (100%) were unpaired, 20 (68.97) aligned zero times, 9 (31.03%) aligned one time and none aligned more than one time. Scaffold 7885 had two different genes running in the same region at opposite orientations.

Gene Match Name	Transcript Name	Orientation	Scaffold	Position	# Exons
GABA-A receptor subunit pi	DN32367_c0_g1_i1	Forward	1	16781435	10
GABA-A receptor subunit rho-1	DN275948_c0_g1_i1	Reverse	77	4638802	3
GABA-A receptor subunit alpha-6	DN43799_c0_g1_i2	Forward	197	2828717	7
GABA-A receptor subunit beta-3	DN40718_c1_g1_i1	Reverse	803	933379	1
GABA-A receptor subunit delta	DN13462_c0_g1_i1	Forward	919	525477	9
GABA-A receptor subunit pi	DN32922_c0_g1_i1	Reverse	4053	33801	8
GABA-A receptor-associated protein	DN9125_c0_g1_i1	Reverse	7885	19681	2
GABA-A receptor-associated protein	DN193150_c0_g1_i1	Forward	7885	20008	2
GABA-A receptor-associated protein	DN4897_c0_g1_i1	Forward	8959	24420	3

Figure 2. – Different GABA receptor gene components. Genes located on the same scaffold at nearby positions are more likely to be inherited together, as crossing over and independent assortment have had less impact on these proteins.

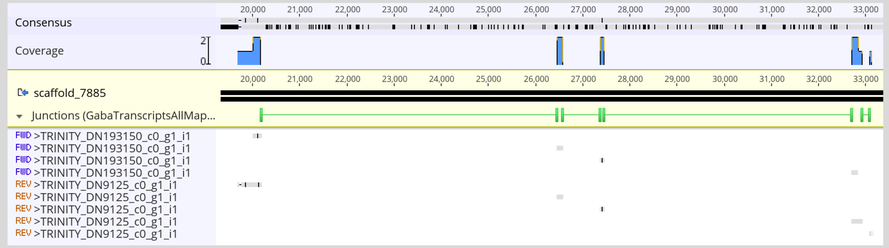

Figure 3. – Two transcripts with overlapping positions in genome. These two GABA-A receptor-associated proteins are present in the same location in the genome but are transcribed in opposite orientations. Their evolution is linked.

Discussion

While research is still ongoing, there is much that we can conclude from the existing data. For example, three genes (those on scaffolds 1, 919, and 4053) have substantially more exons than the others. This is significant because with less coding regions, more DNA sequences are left uncoded, meaning there is a lot of unused genetic data. Furthermore, similarities in the number of exons may signify that the scaffolds are linked and that the two scaffolds indicate shared heritage between the populations. **One intriguing thing I found while conducting my research was two transcripts running along the same position in opposite orientations,** which was unexpected. Upon further research I found that there are similar cases of this occurring in the human genome (Gherman 2009; Chen et al. 2019). This likely means that the two genes that overlap have linked evolutionary histories. On scaffold 8989 I found that despite Frame 3 providing the best match, it still included two stop codons in the read. This likely means that the read is low quality and in the future a long read system like PacBio may be more beneficial. In addition, the low percentage of genes mapping to the genome (31%) indicates that this genome is incomplete and more research needs to be done to allow further analysis amongst the linkage between different GABA transcripts.