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Identifying Dopamine Receptor Genes and Transcription Marbled Crayfish

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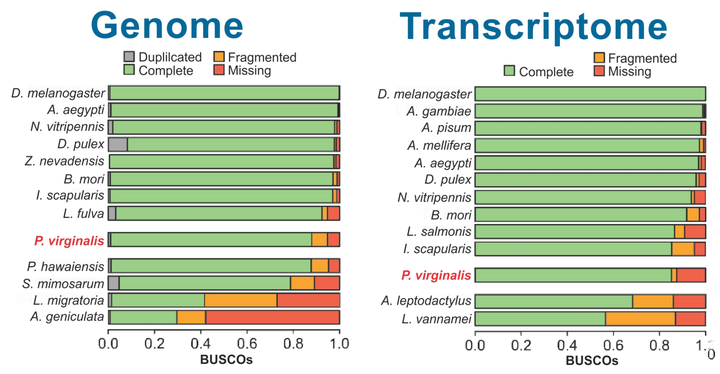
(1) Introduction

Dopamine is a modulatory transmitter and considered a major contributor to nervous system plasticity and behavioral flexibility [1]. Transmitters like Dopamine are either released from neurons or act as hormones. They alter neuronal activity by modifying ionic conductances and synaptic interactions, and thus allow neurons to adapt to changing internal and external conditions [2]. In addition, modulatory transmitters determine motivational states and are involved in psychiatric and neurological disorders in vertebrates and invertebrates [3].

Dopamine acts through a variety of distinct receptors and due to the diversity in receptor subtypes and distribution [3] can exert many different actions in its target tissues. **A prerequisite to understand the ways modulators work is thus to identify which receptors are expressed in an animal.** Despite Dopamine's dominant role in brain modulation, surprisingly little is know about its receptor identities and actions.

(2) Aims

Ideally, to understand Dopamine actions, neuromodulator origins, targets, receptors, molecular pathways, physiological responses, and behavioral implications should be accessible - traits few study organisms offer. **I studied which Dopamine receptors are present in the crayfish *Procambarus virginalis*, a highly invasive species of all female genetic clones with high quality genome and transcriptomes [4]** (Figure below). Their broad behavioral repertoire makes them ideal for studying the actions of neuromodulator receptors.



(3) Approach and hypothesis

Using bioinformatics on the genome and transcriptome, we identified which dopamine receptors exist in marbled crayfish. Two receptors previously identified in nervous tissue of spiny lobsters, **D1Alpha and D1beta** [5], were considered. Like crayfish, lobsters are decapod crustaceans, and the evolutionary closest species with identified dopamine receptors.

I hypothesize that both previously identified crustacean dopamine receptors are also expressed in marbled crayfish.

(4) Which DA receptors exist in marbled crayfish?

We performed BLAST analyses (Uniprot and German Cancer Research Center Marmorkrebs database) against spiny lobster to identify D1Alpha and D1beta receptor homologs in the marbled crayfish (MC) genome.

Gene name	Location in MC genome	Gene length	Bit Score	E-Value	Homology to lobster
D1Alpha	Scaffold 60229	74910 bp	228	1E-57	37%
D1beta	Scaffold 101628	106413	189	2E-46	86%

We identified two putative dopamine receptor homologs by their high Bit Score (>100) and low E-values (near 0).

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4. Gutekunst, J. et al. *Nature Ecology & Evolution*. 2018. 2(3):567-573.
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(5) Which putative dopamine receptors are expressed?

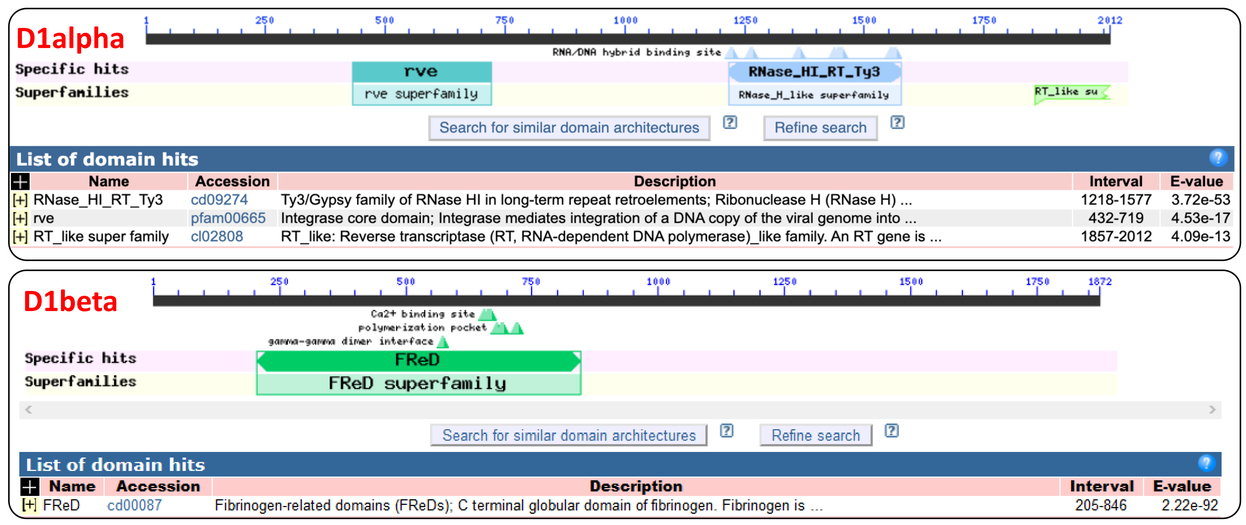
We used BLAST analysis and a search of the marbled crayfish transcriptome to identify transcripts of the two putative dopamine receptors.

We found multiple transcripts for the two putative receptors, indicating that multiple splice variants may be expressed.

Gene name	Transcript name	Bit Score	E-Value
D1Alpha	13991	1072	0
	45	797	0
	64	684	0
D1beta	11175	353	1E-94
	21440	351	4E-94

(6) Do the putative dopamine receptors contain functional domains consistent with dopamine receptors?

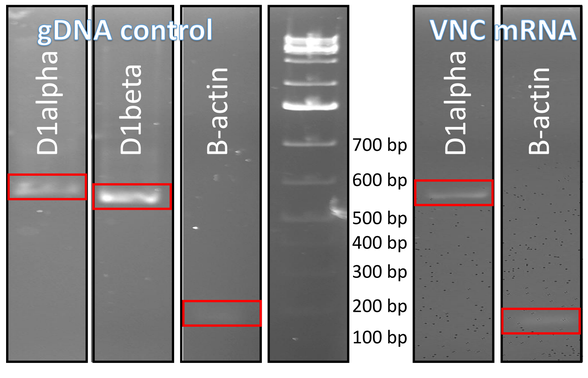
We used a conserved domain database search to determine whether the transcripts identified above share domains with dopamine receptors in other species.



All D1alpha transcripts contained RNAase domains, but no functional domain related to dopamine receptors. Only one D1beta transcript returned a match: fibrinogen-related protein and thus also not a dopamine receptor-related domain.

(7) Are the putative dopamine receptors expressed in the nervous system?

We tested whether the putative receptors are expressed in one of the main nervous system tissues in crayfish, the ventral nerve cord (VNC). We created primer pairs to selectively amplify either D1Alpha or D1beta transcripts from VNC mRNA (predicted lengths: 578 bp) in a PCR reaction. Agarose gel results are shown to the left. mRNA was converted to cDNA for the reaction. B-actin was used as a control (166 bp). Genomic DNA control shows that the created primers amplified sequences of the expected lengths for D1Alpha and beta.



Only D1alpha was used for the VNC PCR. **The presence of a band at the expected size indicates that D1alpha is expressed in the ventral nerve cord.**

(8) Conclusions, discussion and future directions

- We identified homologs of lobster D1Alpha and D1beta dopamine receptors in the marbled crayfish genome and the transcriptome.
- However, we were unable to confirm a functional dopamine receptor domain.
- PCR revealed that at least the putative D1Alpha is expressed in the ventral nerve cord.

While homolog to lobster dopamine receptors, we currently cannot confirm the function of these genes in marbled crayfish. To further assess their functions, we are now testing their expression in other tissues.