The transcriptome of the spider *Cupiennius salei* peripheral nervous system – identifying genes involved in mechanosensation

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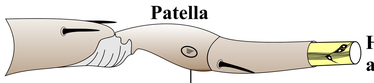
1. Leg hypodermis and VS-3 Organ

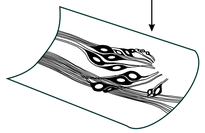
Cupiennius salei is a tropical wandering spider. The leg hypodermis forms a continuous fibrous sheet that attaches to the cuticle at each joint. Leg nerves and many sensory neurons are embedded between layers of the hypodermis.

The cuticle contains many slit sensilla that sense mechanical strain. Each slit is innervated by a pair of sensory neurons.

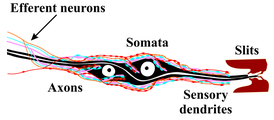
We are using VS-3 slit sensilla in the patella to study mechanotransduction and its modulation by efferent axons from the central nervous system.

This poster describes recent work to identify genes for proteins involved in mechanotransduction and its modulation.





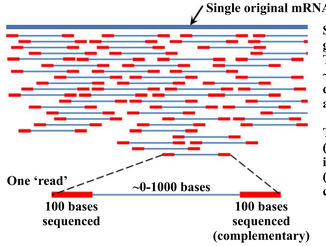
The VS-3 slit-sense organ has a group of 14-16 neurons in the patellar hypodermis that innervate 7-8 slits in the patella.
They can be penetrated by microelectrodes to observe mechanotransduction and other sensory processes.
We have previously used voltage clamp and fluorescent calcium indicators to characterize the mechanically-activated current and its modulation by calcium and other second messengers.
The mechanically-activated current is strongly Na^+ selective, and amiloride-blockable.



Each pair of neurons is surrounded by efferent nerve fibers from the CNS that synapse onto the axons, somata and sensory dendrites. GABA, glutamate, octopamine and acetylcholine have been identified as transmitters. We have also found and characterized several transmitter receptor molecules.

We extracted 8 µg RNA from the leg hypodermis of seven adult female sibling spiders (56 legs) using a Qiagen RNeasy plus mini kit. cDNA library construction and Illumina processing were performed by McGill University and Génome Québec Innovation Centre, Montréal, Québec.

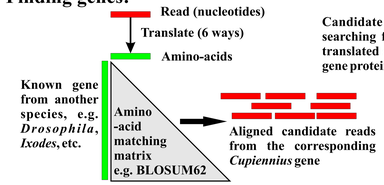
2. Finding genes



Sequencing the hypodermis mRNA gave ~430 million paired-end reads. The average fragment length was ~200 bases. Each base also has a quality score that can be used when assembling genes.

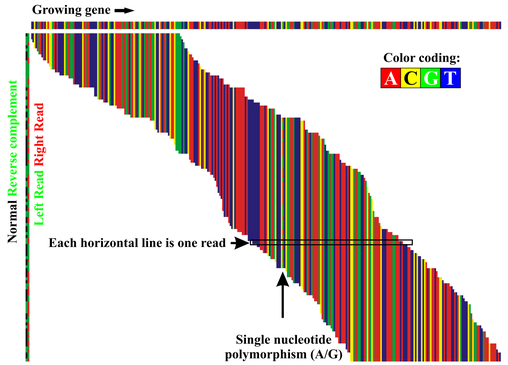
The tasks are:
(1) Find reads from the genes of interest, and
(2) Assemble the complete protein coding sequences for the genes.

Finding genes:



Candidate reads are found by searching for close matches of the translated amino-acids to a known gene protein sequence.

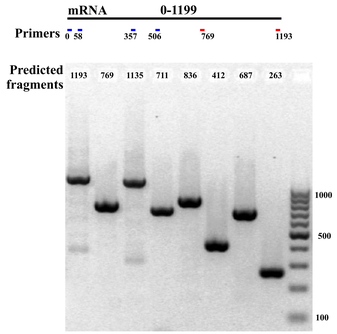
3. Assembling genes



Genes are assembled by 'Transcriptome walking'. The algorithm searches for reads that match the end of the growing gene (typically 60 bases overlap) until enough are found to extend the end with high confidence (typically >20 reads). New reads are melded using majority vote, weighted by the quality scores. Detection of single nucleotide polymorphisms requires allowing 2-3 unmatched bases. Walking is possible in either direction.
More information in: French (2012) BMC Research Notes 5:673-680.

4. Verifying assembled genes

RT-PCR of a putative glutamate-activated Cl^- channel gene



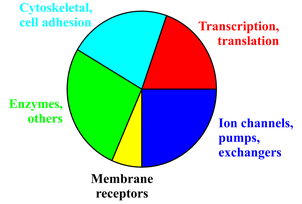
Hypodermis RNA was probed by all combinations of four forward and two complementary 25-base primer sequences to give 8 amplified fragments covering the entire reading frame. All predicted fragments were found.

Putative glutamate-activated chloride ion channel (GenBank GAKT01000005).

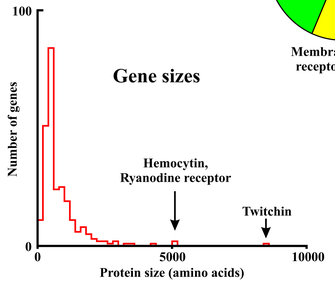
5. Assembled gene properties

Summary properties of 260 genes assembled from the *Cupiennius salei* hypodermis transcriptome

Types of genes

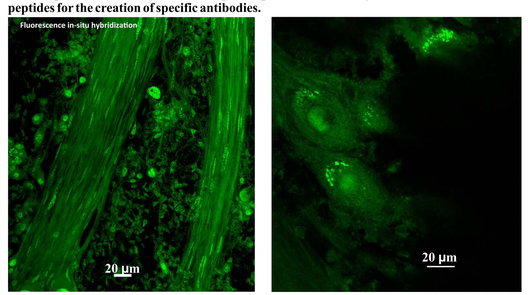


Gene sizes



6. Locating assembled genes

Gene sequences can be used to create specific probes for *in situ* hybridization or translated to peptides for the creation of specific antibodies.



Left: *in situ* hybridization of spider leg nerve using a fluorescein labeled probe for the assembled *Cupiennius* REPO (GenBank GBFC01000008). REPO is a glial-specific nuclear homeobox protein. Labeling of REPO mRNA was strongest in glial cell nuclei.

Right: *in situ* hybridization of VS-3 neuron somata using 10 labeled probes for an assembled *Cupiennius* glutamate-activated chloride ion channel (GenBank GAKT01000005). Strong labeling for mRNA was seen in the endoplasmic reticulum surrounding the nuclei.

Single probe for Left figure (IDT-technologies):
ACGGTGGGGGTCGACATCTTGAGTATCCAACCCAACTAATCCCGTCATTTTCTT
GTCGGTGCCAGAACCGTTACCACCATTTCCGTAGAC

7. Findings and future directions

More than 260 genes have been identified and sequenced so far.

Genes that could be involved in mechanotransduction:
(1) ENaC/ASIC/DEG (amiloride-blockable ion channels) (x3)
(2) TRP ion channel
(3) Piezo ion channel
(4) Echinoderm-microtubule-related (ciliary basal body protein)

Genes that could be involved in modulation of mechanosensation:
(1) Acetylcholine channels (both nictonic and muscarinic)
(2) Dopamine receptors (x2)
(3) GABA channels (x5)
(4) Glutamate-activated Cl^- channels (x4)
(5) Octopamine receptors (x5)
(6) Serotonin receptor
(7) Voltage-gated Ca^{2+} channels, Ca^{2+} signaling proteins and Ca^{2+} buffers (all numerous)

Genes that could be involved in action potential signaling and modulation:
(1) Voltage-activated K^+ and Na^+ channels (several of each)
(2) Ca-activated K^+ and Cl^- channels

Future Directions (all in progress)

(1) *in situ* hybridization to locate which cells express genes of interest
(2) Creation of custom antibodies to locate cellular regions of protein activity
(3) RNA interference to test functional roles of individual genes

See this poster again at: http://asf-pht.medicine.dal.ca