MOL231: Identifying Candidate Genes to Visualize the Unique Ctenophore Nerve Net

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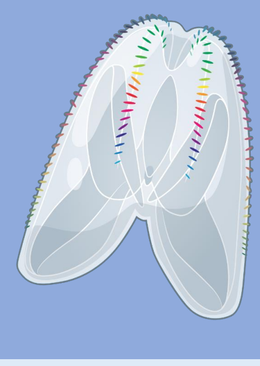

Background and aims

Ctenophores are marine invertebrates that are strong candidates for being the sister group to all other animals (1). These animals have a nerve net that is anatomically and molecularly distinct from other neurons. They are therefore a good model organism to study the **evolution of nervous systems.** The connections between five different nerve net neurons were found to have a continuous plasma membrane, they had no electrical or chemical synapses between them at the cydippid stage (2). This so called **syncytial nerve net** raises several questions as to how this nerve net develops, grows and functions. There are few nerve net markers available and no functional tools to study the nerve net *in vivo.*

In this project we aimed to identify candidate genes to generate **transgenic lines** or **knock-ins** in the ctenophore ***Mnemiopsis leidyi*** to be able to study the development of this nerve net *in vivo.*

Methods

Existing scRNAseq data (3) was used to select genes with a high fold change in the nerve net clusters.

Hybridization chain reaction (HCR) was performed on whole mount animals to analyze the expression patterns. Existing nerve net markers for neuropeptides ML199816a and ML02212a were used to confirm their specificity (4).

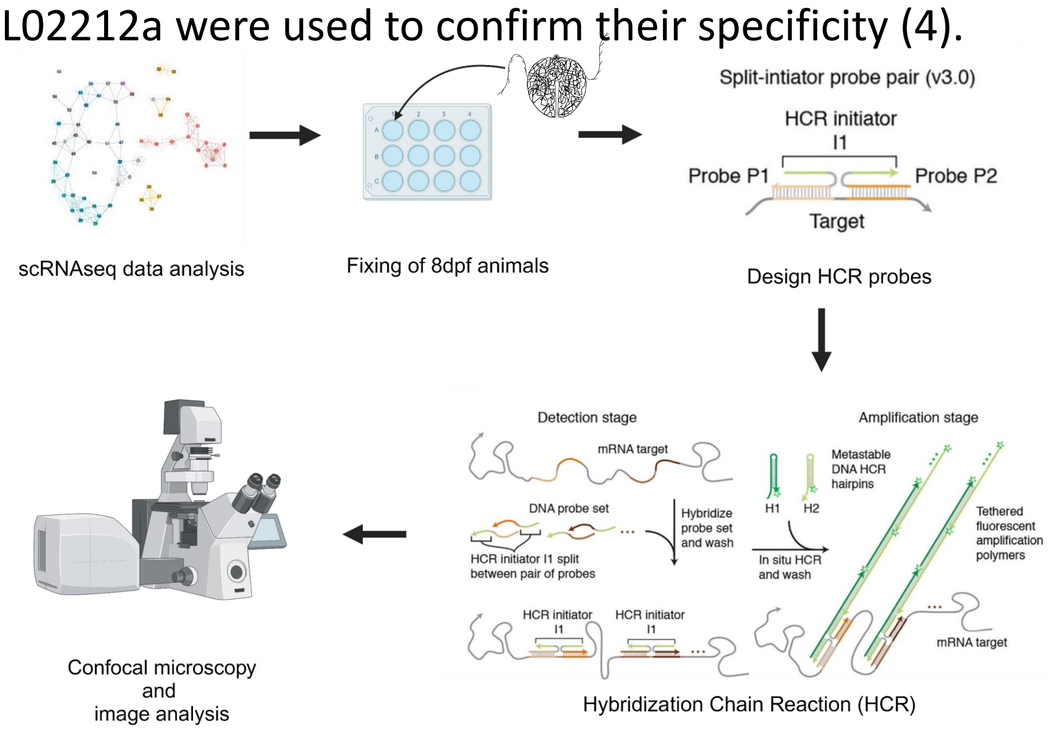

Figure 1: Overview of method work-flow (5). Image created with BioRender.

Results

- We identified genes with a high fold change in clusters 49 and 50 which could be used as potential candidates for generating reporter lines.
- Background fluorescence is present mainly in the tentacle bulbs in the 488 and 546 excitation wavelengths.
- The neuropeptide ML02736a is expressed in the nerve net surrounding the comb rows (Fig. 3).
- Ankyrin repeat and SAM domain-containing protein 6 (ANKS6) is highly expressed in the comb rows and in the AO floor. Some co-expression with nerve net markers was also observed (Fig. 4).

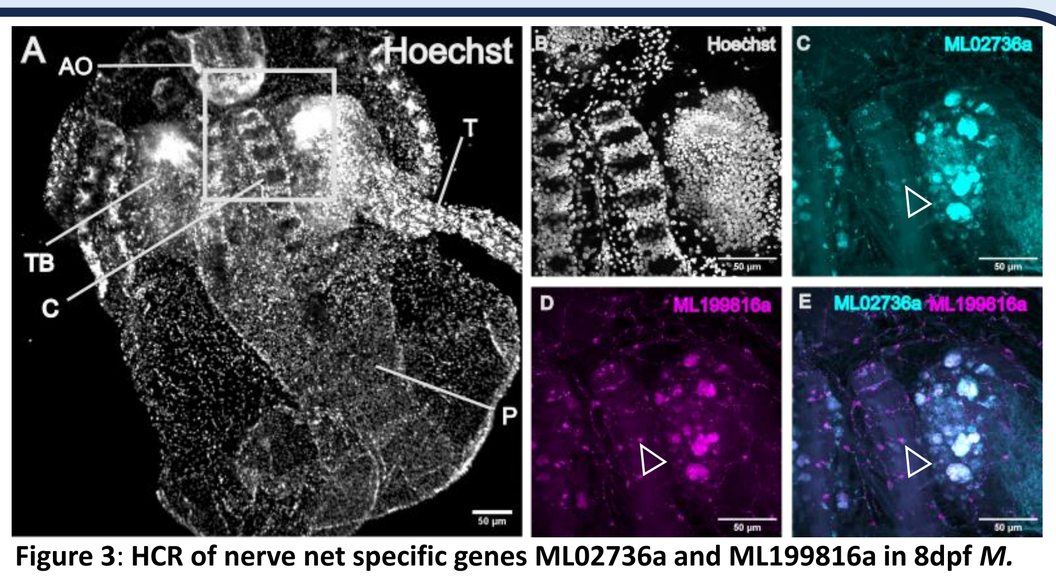

Figure 3: **HCR of nerve net specific genes ML02736a and ML199816a in 8dpf *M. leidyi***. A. Nuclear staining with Hoechst shows an overview at 20x, white box indicates magnified region at 60x shown in B-E. B. 60x, nuclei stained with Hoechst. C. mRNA expression of neuropeptide ML02736a. D. mRNA expression of nerve net marker neuropeptide ML199816a. E. Co-localization of both genes in the same cells can be observed in the comb rows. AO, aboral organ; TB, tentacle bulb; C, comb rows; T, tentacle; P, pharynx.

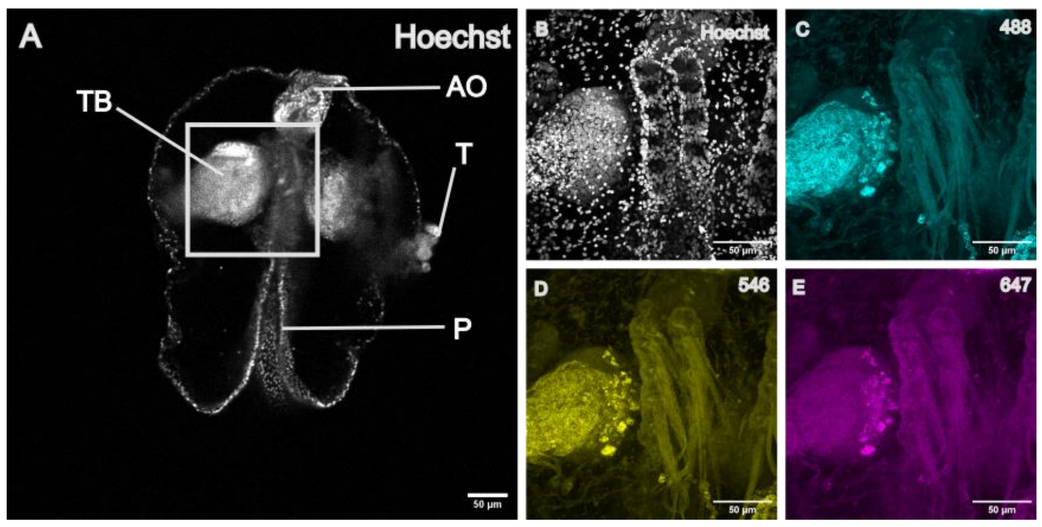

Figure 2: **HCR without probes as a control in 8dpf *M. leidyi***. A. Nuclear staining with Hoechst shows an overview at 20x, white box indicates magnified region at 60x shown in B-E. B. Nuclei stained with Hoechst. C-E. Background fluorescence at excitation wavelengths 488, 546 and 647. AO, aboral organ; TB, tentacle bulb; T, tentacle; P, pharynx.

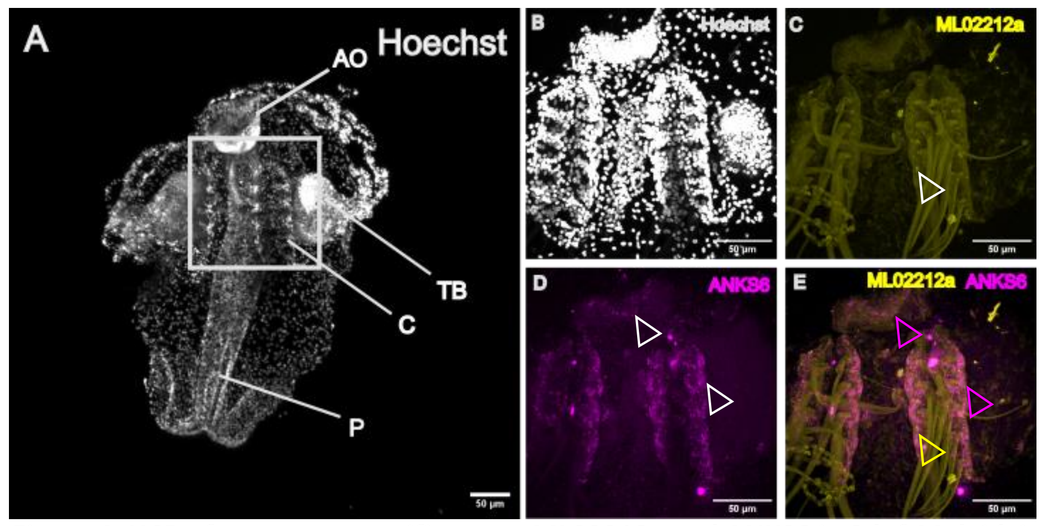

Figure 4: **HCR of nerve net specific genes ML02212a and ANKS6 in 8dpf *M. leidyi***. A. Nuclear staining with Hoechst shows an overview at 20x, white box indicates magnified region at 60x shown in B-E. B. 60x, nuclei stained with Hoechst. C. mRNA expression of nerve net marker neuropeptide ML02212a. D. mRNA expression of ANKS6. E. Co-localization of both genes in the same cells can be observed in the comb rows. AO, aboral organ; TB, tentacle bulb; C, comb rows; P, pharynx.

Conclusion and Future work

ANKS6 is also expressed in other cell types whereas the neuropeptide ML02736a could be used as a candidate gene to generate reporter lines.

References

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