MOL231: Unravelling the Role of *Netrin* in Axon Guidance: Insights from the Nervous System of *Nematostella vectensis*

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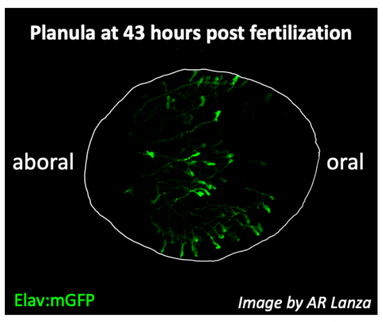
Photo: Chiara Sinigaglia

Background

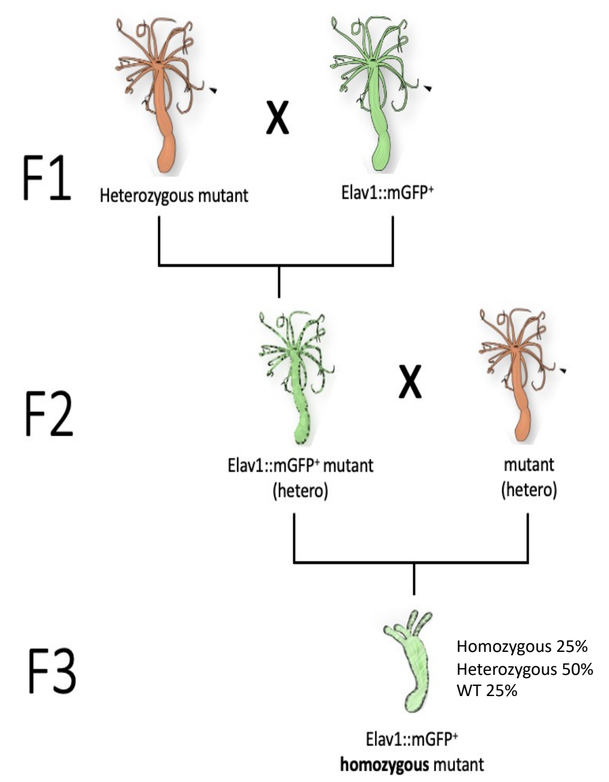
The formation of a nervous system relies on the establishment of connections between neurons (1). These connections are formed by axonal outgrowths. Netrins are a family of extracellular proteins known to be involved in directing growing axons toward their target (2). However its role in axon guidance in simple nerve nets, like that of *Nematostella vectensis*, is poorly understood.



Early neurites grow in an aboral direction

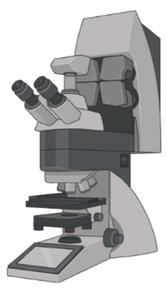
The *Nematostella* nervous system is non-centralized, meaning it has no single brain or central nervous system, but rather a nerve net distributed throughout the body (3). Directional neurite outgrowth can be observed in early neurogenesis of these animals, but the mechanism is not yet known. We inhibited *Netrin* using CRISPR-Cas9 to see if it has a role in neurite outgrowth during early neural development in *Nematostella*. The *Netrin* mutant lines were crossed with an Elav1::GFP line which allows the live visualization of the *Nematostella* nervous system as early as 36 hours post-fertilization.

AIM: genotype *Netrin* CRISPR-Cas9 mutants and analyse neural phenotype

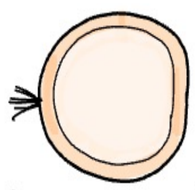


Method

1. Confocal imaging planula at 42 hpf

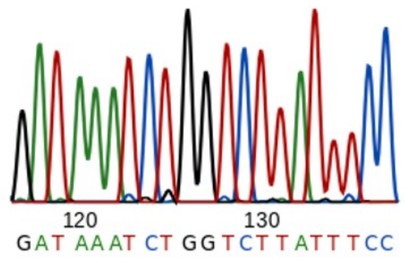


2. Genomic DNA (gDNA) extraction


planula were recovered from slides

3. PCR amplification of mutation site

4. Sequencing



Results

WT

Sequence peaks

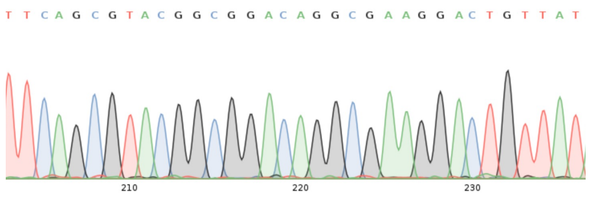


Figure1.1: Uniformed sequence peaks indicate homogeneity between the sequence of both alleles

Nucleotide sequence

```
Template  AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
allele 1  AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
allele 2  AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
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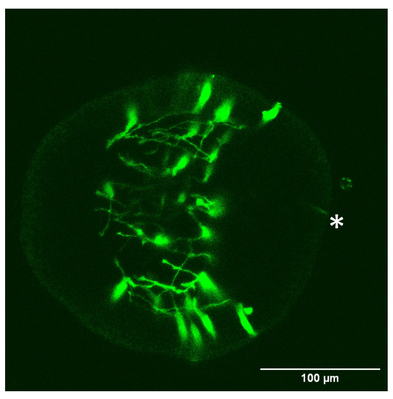
Live fluorescent imaging of Elav1::GFP animals n=9



A — O*

Heterozygous

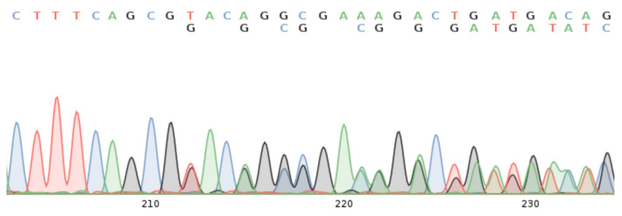
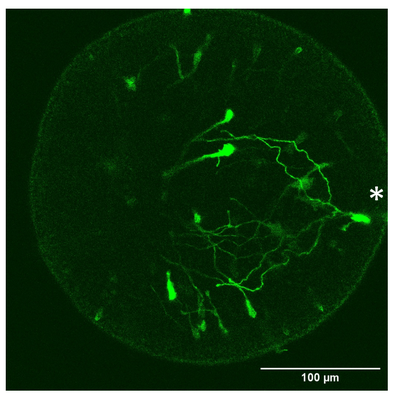


Figure 1.2: Doubled sequence peaks indicate differences between the sequence of each allele.

```
AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
AGGGCTGCGTCTCAATACTTTCAGCG-------GACAGGCGAAGGACTGTTATAAC
```

n=17



Homozygous

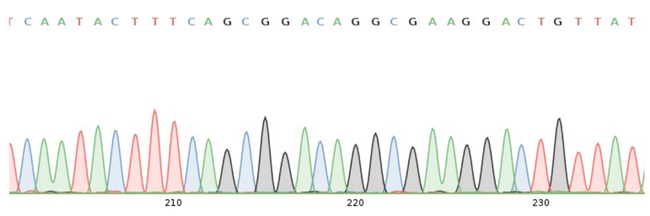
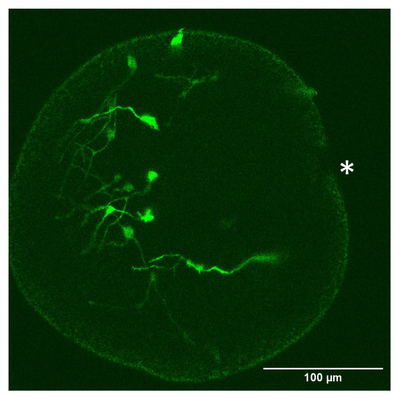


Figure 1.3: Uniformed sequence peaks indicate homogeneity between the sequence of both alleles.

```
AGGGCTGCGTCTCAATACTTTCAGCGTACGGCGGACAGGCGAAGGACTGTTATAAC
AGGGCTGCGTCTCAATACTTTCAGCG-------GACAGGCGAAGGACTGTTATAAC
AGGGCTGCGTCTCAATACTTTCAGCG-------GACAGGCGAAGGACTGTTATAAC
```

n=15



References

1. Hasegawa K, Kuwako K-i. Molecular mechanisms regulating the spatial configuration of neurites. Semin Cell Dev Biol. 2022;129:103-14.
2. Boyer NP, Gupton SL. Revisiting Netrin-1: One Who Guides (Axons). Front Cell Neurosci. 2018;12:221.
3. Nakanishi N, Renfer E, Technau U, Rentzsch F. Nervous systems of the sea anemone Nematostella vectensis are generated by ectoderm and endoderm and shaped by distinct mechanisms. Development. 2012;139(2):347-57.

SCAN ME

Conclusion

1. Homozygous *Netrin* mutants containing a 7 bp deletion were successfully generated using CRISPR-Cas9.
2. We observed 22% wild type, 41% heterozygous mutants, and 37% homozygous mutants after genotyping the F3 generation. This did not match the inheritance probability, but may be due to our small sample size.
3. Phenotypically, when compared to control, we observed a reduced number of neurons and neurites in heterozygous animals with even more reduction in homozygous animals as well as changes in spatial distribution of neurons. However, no changes in neurite direction were observed.
4. In order to gain a more comprehensive understanding of the patterns of neurite change in homozygous animals, further live imaging is required.