RING Finger Proteins in Crossover Designation and Interference

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Overview

- Vilya, Narya, Nenya (VNN) proteins aid in double-strand break (DSB) formations and crossovers
- Higher expression of these proteins is predicted to increase crossovers in meiosis, decreasing interference
- A transgene containing VNN was constructed to regulate gene expression to test our hypothesis
- The VNN transgene was followed in Drosophila crosses and an increase in crossovers was found
- Individual deletions of Narya and Nenya will also be made

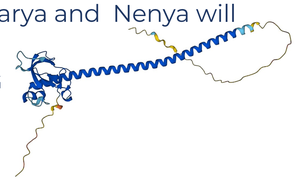


Figure 1. Image of the RING finger protein Vilya

Introduction

- Crossovers are required for segregation of chromosomes in meiosis; Without this process, nondisjunction may result which can result in certain chromosomal conditions (4)

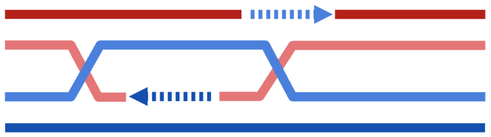


Figure 2. Double Stranded Breaks allow for proper chromosome segregation

- Vilya is found within the synaptonemal complex (SC), a structure formed during meiosis before chromosome segregation
- It interacts with Mei-P22 to form DSBs (2)
  - Sufficient Vilya is needed for crossovers to occur

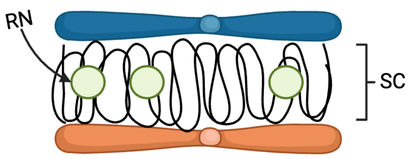


Figure 3. Vilya, Narya, and Nenya are found in recombination nodules (1, 2)

- Narya and Nenya have also been implicated in crossover formation in the SC
  - They have been found to colocalize and interact with Vilya (1) in recombination nodules (RN) within the SC

This information indicates a role for VNN in crossover designation

Methods

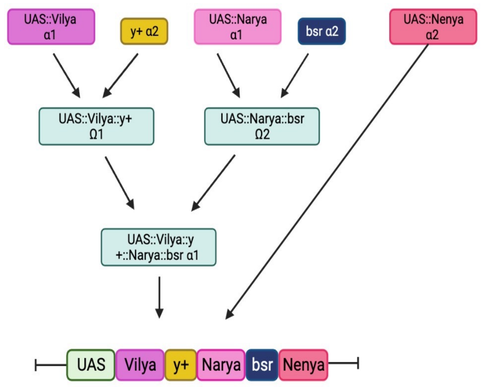


Figure 4 (left). Flowchart depiction of UAS::VNN assembly done via Goldenbraid cloning

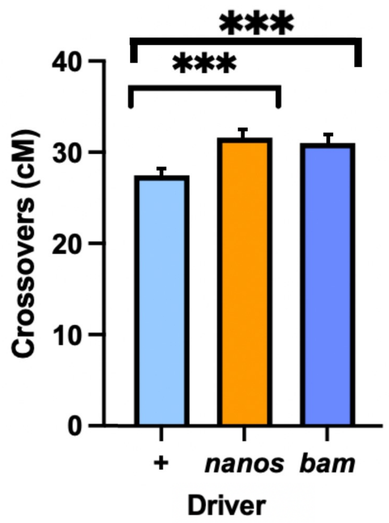
- This construct was put into an aattB vector and then sent for injection into Drosophila
- Drosophila containing UAS::VNN transgene were crossed with Drosophila containing GAL4 drivers to activate gene expression

(UAS::VNN @ 6E)/Fm7 ; hodpPb/CyO
x
P{YFG::GAL4} on the X

- Females were collected and crossed with yw^1118 males and then screened for crossovers

Results

- Scored UAS::VNN Drosophila amplified with GAL4::nanos & GAL4::bam
- Compared against WT data and a negative control cross
- n = 17713



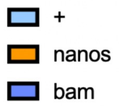
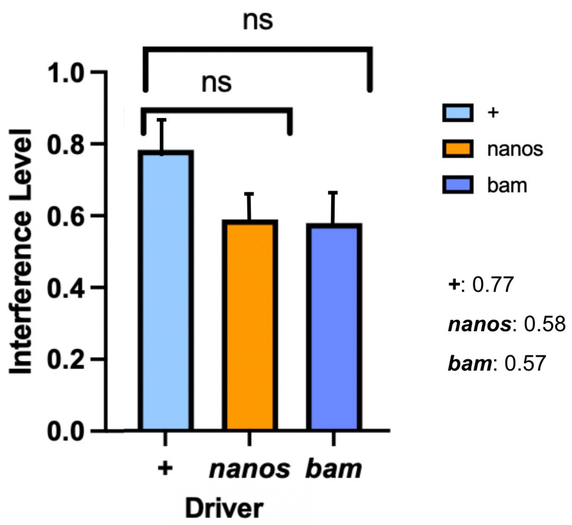


Figure 5 (left). Crossover Levels for UAS::VNN Drosophila vs. WT

- VNN x bam = 111.2% WT
- VNN x nanos = 113.3% WT
- VNN x yw = 98.6% WT

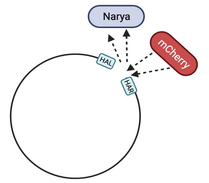
Figure 6 (right): Interference levels between UAS::VNN Drosophila and WT

- We believe a larger sample size would produce statistically significant results when compared to WT



Future Directions

- Narya and Nenya will be looked at individually
  - Marked deletions will be made using CRISPR/Cas9
  - Narya ⟶ mCherry ; Nenya ⟶ GFP; Vilya ⟶ GFP
  - Narya and Nenya believed to be redundant
- UAS::VNN will be looked at with a mei-218 null
  - Critical for crossover formation (3)
- To get quantitative data on the levels of UAS::VNN expression, qRT-PCR will be performed on Drosophila ovaries



- **Figure 7**. Depiction of replacement of Narya with mCherry , creating a marked deletion

Conclusions

- Crossover screening in Drosophila has revealed that there is a statistically significant increase in crossovers in Drosophila containing the UAS::VNN transgene, indicating that the three genes have a role in crossover designation
- While our sample size showed no significance in interference, it is likely that with a larger sample there would have been a significant decrease in crossover interference based on current knowledge of how designation and interference interact

Acknowledgements

- Members of the Sekelsky Lab

References

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