Insertion of WrmScarlet11 into cosa-1 gene in C. elegans using CRISPR

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Background/Introduction

The C elegans germline is a U-shaped tissue with mitosis occurring distal from the uterus and meiosis proceeding as cells move proximally, as seen in the drawing above. Meiosis encompasses a number of different stages: pairing of chromosomes, crossover formation, chromosome remodeling, and germ cell maturation. During crossover formation, COSA-licences crossovers between paired chromosomes. To visualize crossovers in vivo, I attempted to insert part of a fluorescent protein into cosa-1.

Shown above is the fluorescent protein wrmScarlet as viewed from the front (A) and top (B). It is made up of 11 beta strands and a fluorescent functional group. In order to fluoresce, all the beta strands must be present in a beta barrel shape. However, beta strand 11 (rainbow) can be on a different protein and still form the beta barrel with 1-10. Therefore, we can tag COSA-1 with the 11th strand in an animal that expresses wrmScarlet10 constitutively, localizing fluorescence to COSA-1, and therefore to crossover sites. To insert the 11th beta strand into the cosa-1 gene, I used CRISPR.

CRISPR-Cas9 can locate a target DNA sequence using a guide RNA and cut it. This induces the cell to repair its DNA. There are two types of DNA repair: NHEJ and HDR. While NHEJ just sticks the broken ends of DNA back together, HDR looks for donor DNA that is similar around the cut region and uses it as template for repair. By making a repair template that includes wrmScarlet11 along with some homologous sequence to the cosa-1 gene, HDR uses it as donor DNA and inserts wrmScarlet 11 into cosa-1, as shown below.

Results

First, into the distal arm of the germline, I injected a mixture containing CRISPR-Cas9 protein and a guide RNA and repair template for cosa-1. As part of a coCRISPR strategy, the injection mixture also contained a guide RNA and repair template for dpy-10 which would produce a mutation which results in rollers when heterozygous and dumpy rollers when homozygous. I incubated the injected worms and waited for progeny (F1).

(A) A precise injection into the germline of C. elegans. The injection mixture is injected into the distal arm of the germline and will disperse evenly in opposite directions, like shown with the arrows. (B) A photo of me performing precise and quick injections and adjusting the focal plane to distinguish the germline.

COSA-1 sequence (right, rainbow) was injected into the wrmScarlet11 sequence (top, red). To visualize crossovers in vivo, I attempted to insert part of a fluorescent protein into cosa-1.

Progeny (F1) that were rollers and dumpy had been CRISPR-edited for dpy-10 in the mother, so they were cloned into individual plates to determine whether they were also CRISPR-edited for cosa-1. Then I incubated the F1s and waited for more progeny. I genotyped the F1s and found that six lines had potential insertions. Progeny of these F1s were followed up.

Progeny from F1 (F2s) were cloned out, incubated and then genotyped. Four F2 worms contained a single white mark between the 600bp and 700bp suggesting that they were homozygous.

I looked at the four homozygous lines, and I saw more males than wildtype. This phenotype hinted at a loss of function in cosa-1. Since cosa-1 is required for crossovers, and crossovers hold the paired chromosomes together for the first division, an absence of cosa-1 might result in chromosome nondisjunction. This may in turn result in some progeny receiving one X chromosome, resulting in males. This suggested that the insertion may not be as I had planned, so I prepared to sequence the 4 homozygous lines.

Conclusions

• I got worms with insertions in cosa-1
• A frame shift occurred due to the deletion of two nucleotides resulting in loss of function in cosa-1
• Loss of function in cosa-1 led to having more males
• Some isolates had a nucleotide change resulting in a missense mutation

Future Directions

• We will determine why the insertion was not as we expected.
• We will inject with corrected injection mix and screen to generate the correct insertion.

Acknowledgements

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References

1) Yokao et al. Cell 2012; 149:75-87
2) Gao et al. Genetics 2021; 217: 4
3) Pau et al. Genetics 2015; 201:1:47-54