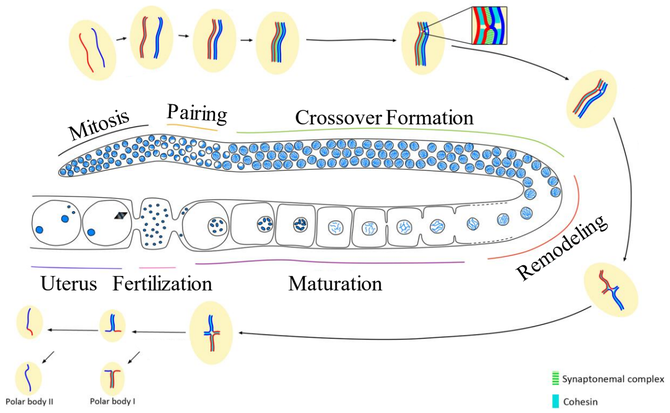
Insertion of WrmScarlet11 into *cosa-1* gene in *C. elegans* using CRISPR

Jose Araujo[1,2], Kenny Trimmer[1], Swathi Arur[1]

[1]University of Texas MD Anderson Cancer Center, Genetics Department
[2]University of Texas MD Anderson Cancer Center, Faculty Diversity, Equity and Inclusion Department

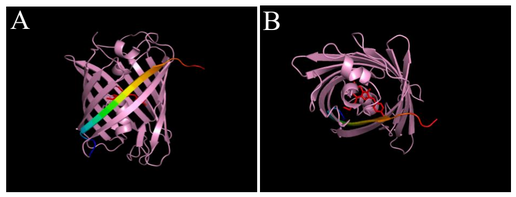
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Background/Introduction

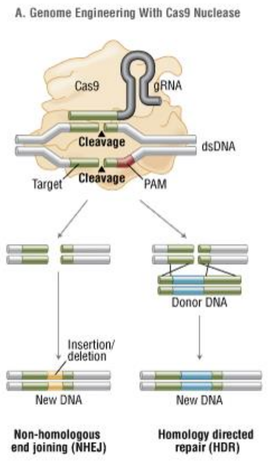


Adapted from http://www.wormbook.org/chapters/www_meiosis/meiosis.html

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-1licences crossovers between paired chromosomes[1]. To visualize crossovers in vivo, I attempted to insert part of a fluorescent protein into *cosa-1*.

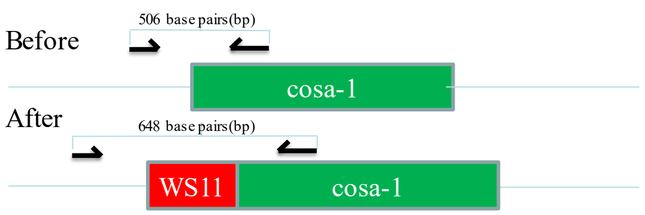


Shown above is the fluorescent protein wrmScarlet as viewed from the front (A) and top (B). It is made up of 11 beta strands and a fluorescent functional group. In order to fluoresce, all the beta strands must be present in a beta barrel shape. However, beta strand 11 (rainbow) can be on a different protein and still form the beta barrel with 1-10[2]. Therefore, we can tag COSA-1 with the 11th strand in an animal that expresses wrmScarlet1-10 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 .



https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

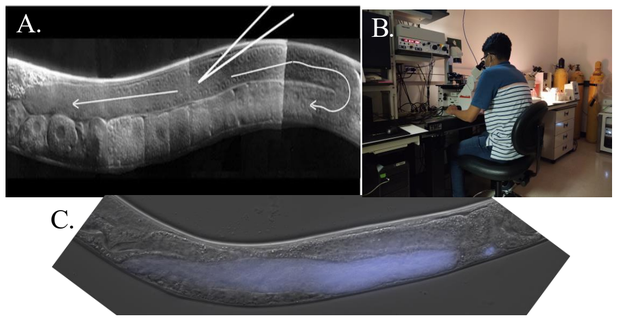
CRISPR-Cas9 can locate a target DNA sequence using a guide RNA and cut it. This induces the cell to repair its DNA. There are two types of DNA repair: NEHJ and HDR. While NEHJ just sticks the broken ends of DNA back together, HDR looks for donor DNA that is similar around the cut region and uses it as 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.



The desired insertion of wormScarlet11 (red) into the cosa-1 gene (green) is shown above. The primers used for genotyping and sequencing are shown by the arrows and the expected band lengths are written above the primers.

Results

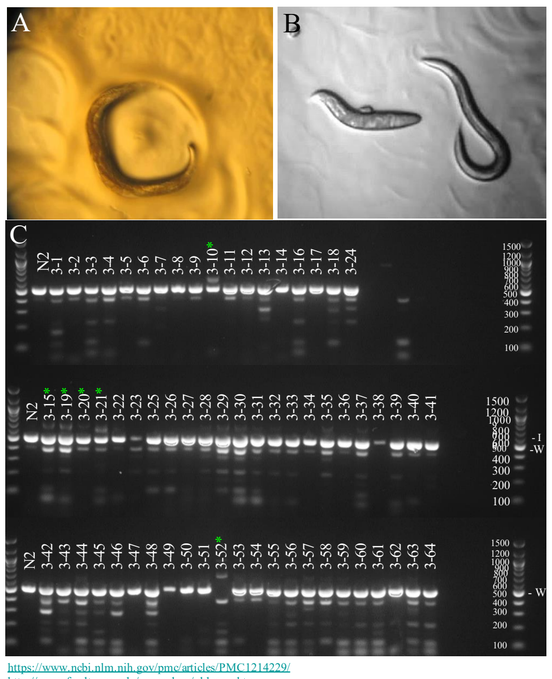
First, into the distal arm of the germline, I injected a mixture containing CRISPR-Cas9 protein and a guide RNA and repair template for *cosa-1*. As part of a coCRISPR strategy, the injection mixture also contained a guide RNA and repair template for dpy-10 which would produce a mutation which results in rollers when heterozygous and dumpy rollers when homozygous[3]. I incubated the injected worms and waited for progeny (F1).



http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html

(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.(C) A successful injection into the germline using a fluorescent label (blue) to visualize the result of an injection.

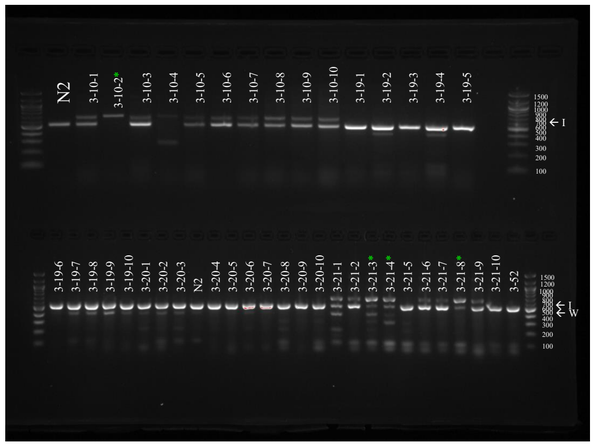
Progeny(F1) that were rollers and dumpys had been CRISPR-edited for dpy-10 in the mother, so they were cloned into individual plates to determine whether they were also CRISPR-edited for *cosa-1*. Then I incubated the F1s and waited for more progeny. I genotyped the F1s and found that six lines had potential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



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1214229/
http://www.faculty.ucr.edu/~mmaduro/oldnews.htm

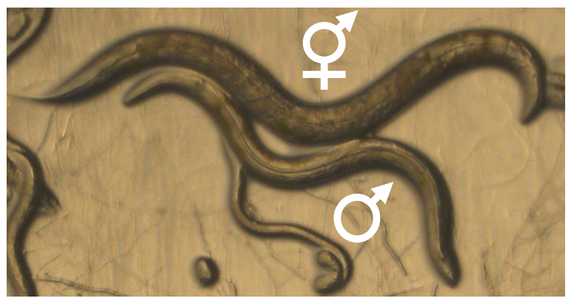
(A) This worm displays a rolling phenotype (a helically twisted body). This worm is identifiable by its rolling movement and the circle traces that it leaves behind. (B) This picture is showing a comparison between a dumpy(left, short and wide) and a regular wildtype worm (right, long and skinny). (C) Worms cloned out from plates containing dumpys and rollers were ran on the depicted gel. Worms marked with a star(*) appeared to be heterozygous/homozygous for wrmScarlett. The ladder is labeled with different band lengths and the length of an insertion (I) and a wildtype worm (W) are also included.

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.



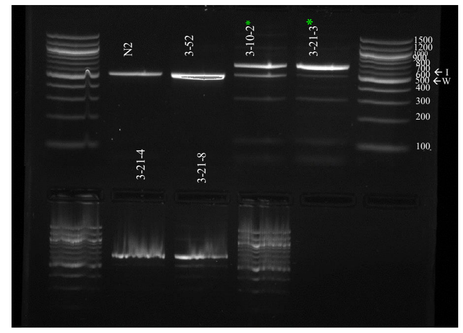
Gel imaging of the second generation of worms (F2s) that were cloned from F1 worms that demonstrated a wrmScarlet11 insertion. A total of 43 samples were screened to detect a band length of 648 base pairs. The labels that include a star (*) are the ones that showed promising band length due to bright white marks appearing in the 600-700bp ladder length.

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[1]. 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.



A comparison of a hermaphrodite (top, slim tail) and a male (middle, blunt tail with fan) found in my cloned-out plates.

From each homozygous line of worms, I amplified the DNA in preparation for sequencing. Since most of the lines had multiple DNA bands and we were only interested in the 648bp band, the PCR product of each line was run on a gel, the correct band was cut out, and the DNA was purified before sending it for sequencing.



Gel imaged from large scale PCR of worms that were promising to have the wrmScarlet11 insertion. These worms were picked out from plates of F2s that appeared to have the insertion. The ones that have a star (*) label on them were thought to be homozygous for wrmScarlet11.

I obtained the results from the worms that were sequenced. In 3 of them a missense mutation occurred. However, in all of them I saw the deletion of two nucleotides. This would result in a frame shift, changing the amino acid sequence and confirming that we had produced a loss of function.

Sequencing results from our 6 promising worms. In the insertion site, there was a gap in the sequence traces; thus, demonstrating the absence of nucleotides(left rectangle), and a nucleotide change was also apparent in some sequences(right rectangle).

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 reinject with corrected injection mix and screen to generate the correct insertion.

Acknowledgements

Thank you to my mentor Kenny for helpful discussions

References

1) Yokoo et al. Cell 2012; 149:75-87
2) Goudeau et al. Genetics 2021; 217: 4
3) Paix et al. Genetics 2015; 201:1:47-54