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Using Chimeric Proteins to Determine Basis of FBF-2 Localization

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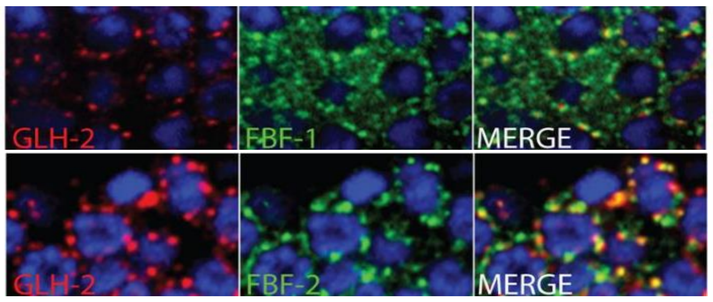
ABSTRACT

FBF-1 and FBF-2 are proteins within the PUF protein family that are required for stem cell maintenance in *Caenorhabditis elegans*. FBF-1 and FBF-2 exhibit mRNA binding activity and are involved in localization, activation and repression of their target mRNA's. The two are similar in sequence with the exception of four Variable Regions (VR's). FBF-2 localizes to P granules in germ cells of the *C. elegans* while FBF-1 does not. We propose that the different localization patterns exhibited between the FBF-1 and 2 are due to these VR's. Analysis of which VR or combination of VR's is responsible for this difference in localization was undertaken through chimeric protein assembly and insertion into the *C. elegans* genome. Assembly of DNA encoding chimeric proteins with various VR's and a tagging Fluorescent Protein (GFP) present was achieved through fusion Polymerase Chain Reaction (PCR) and BP/LR clonase plasmid assembly. Introduction of chimeric DNA constructs is in progress, and done through Crispr-CAS-9 genome editing. Expression of the modified proteins and assessment of localization patterns will be carried out using GFP visualization. The poster will discuss our observations and preliminary conclusions.

INTRODUCTION

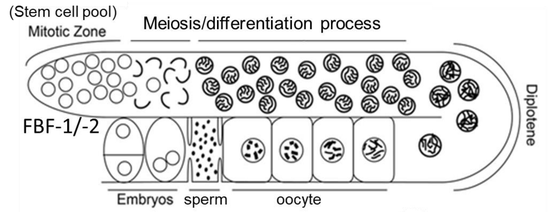
- FBF proteins provide maintenance of *C. elegans* stem cell germline
- The mechanisms through which these two proteins segregate are unknown but they have been found to have different regulatory effects despite their highly similar sequences
- FBF-1 action alone results in an extension of the germ line
- FBF-2 action alone results in a shortening of the germ line and localizes to P granules



Differential FBF protein localization (*Voronina et. al 2012*)

METHODS

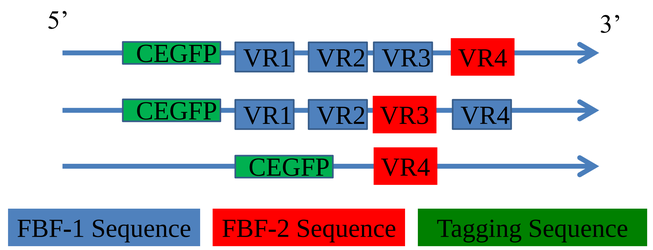
- Fusion PCR
- BP and LR clonase plasmid assembly
- CRISPR gene editing
- Fluorescence and Confocal microscopy



Schematic germline of *C. elegans* hermaphrodite. (*VanGompel et al., 2015)*

FIGURE 1- *C. elegans* Biology

- The *C. elegans* distal germline is composed of mitotic and meiotic zones
- Stem cell proliferation occurs at the very tip of the mitotic region
- As cells mature, expression of proteins changes to develop oocytes and sperm



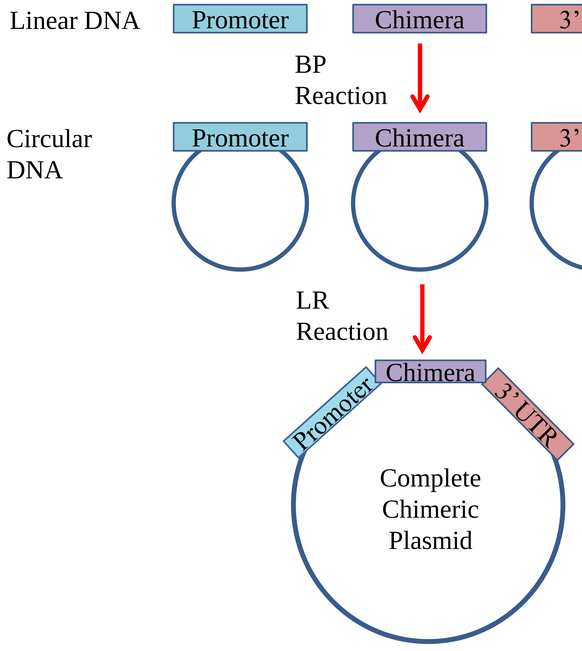


FIGURE 2- Chimeric Protein Sequences and Integration Into *C. elegans* Genome

- Chimeric sequences are constructed to each include one of the FBF-2 VR's
- Chimeric FBF proteins are constructed through fusion PCR reactions that anneal identical segments of DNA together to combine fragments of different *fbf* sequences
- Fluorescent tags are added to chimeric sequences to allow for microscopy analysis
- Linear DNA synthesized through fusion PCR and PCR is introduced into plasmids through BP clonase reactions which allow for transformation and propagation
- BP donor plasmids are then assembled into a coherent protein sequence along with a promoter region and 3' Untranslated Region (UTR) through an LR clonase reaction
- Prepared chimeric sequences are then inserted into *C. elegans* genomes through CRISPR gene editing and screened to determine the effect of the modified sequence

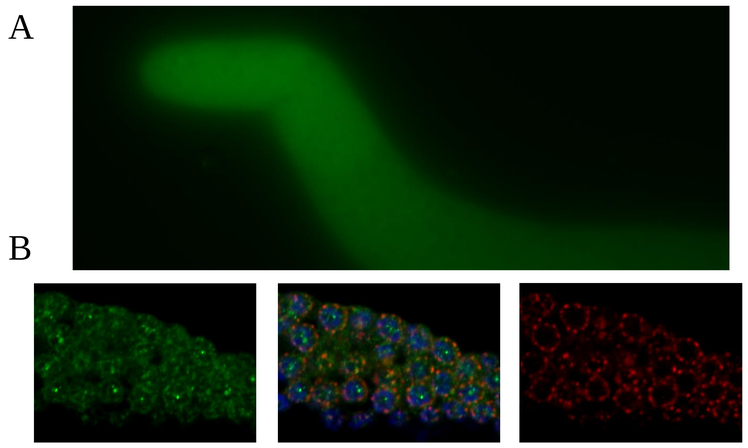


Figure 3- (A) Preliminary Results and (B) Immunostaining

- Fig. 3A
- The CEGFP VR4 chimera is seen above to be localized to the mitotic region of cells and is disposed of as cells transition into the zone of meiosis
- These results indicate that FBF-2's VR4 has some role in the overall localization of FBF-2 and provide an ideal starting point to explore collaborating VR's
- Fig. 3B
- Immunostaining is used to determine patterns of expression for targeted proteins
- Staining provides the location of nuclei (DAPI-the blue), P granules (red) and tagged target proteins (GFP-green) and allows for target protein location in relation to P granules and cell nuclei
- Above, the CEGFP and FBF-2 VR4 chimera can be seen partially localizing to P granules indicating that VR4 is part of the localization sequence of FBF-2
- This assay will be preformed to closely monitor the localization trends of chimeric proteins and understand the mechanisms of FBF regulatory patterns

DISCUSSION

- Chimeric protein synthesis and observation will provide a more nuanced understanding of stem cell regulation and the mechanics of their differentiation
- As new chimeras are produced and inserted into the *C. elegans* genome we can use these genes to study other methods of pots-transcriptional regulation

FUTURE DIRECTIONS

- Expanding knowledge of stem cell regulation allows for further understanding of how to harness them for therapeutic efforts

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- VanGompel, M. J. W., Nguyen, K. C. Q., Hall, D. H., Dauer, W. T., & Rose, L. S. (2015). A novel function for the *Caenorhabditis elegans* torsin OOC-5 in nucleoporin localization and nuclear import. *Molecular Biology of the Cell*

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