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Transgenerational Sterility in *fbf-1* and *rrf-1* Mutant *Caenorhabditis elegans*

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1. Introduction

- The interaction between RNAs and RNA-binding proteins (RBPs) is an important topic in studies of gene expression.
- *fbf-1* and *fbf-2*: genes that encode RBPs that maintain stem cell proliferation and differentiation in the *Caenorhabditis elegans* (*C. elegans*) germline.
- *rrf-1:* a gene that encodes an RNA-polymerase that generates small regulatory RNAs.
- We suspected that at 24° C, a strain of *C. elegans* with *rrf-1* and *fbf-1* mutations becomes sterile over the course of multiple generations.
- Understanding the link between small regulatory RNAs and RBPs is important because their interaction is implicated in many human diseases, including cancer.

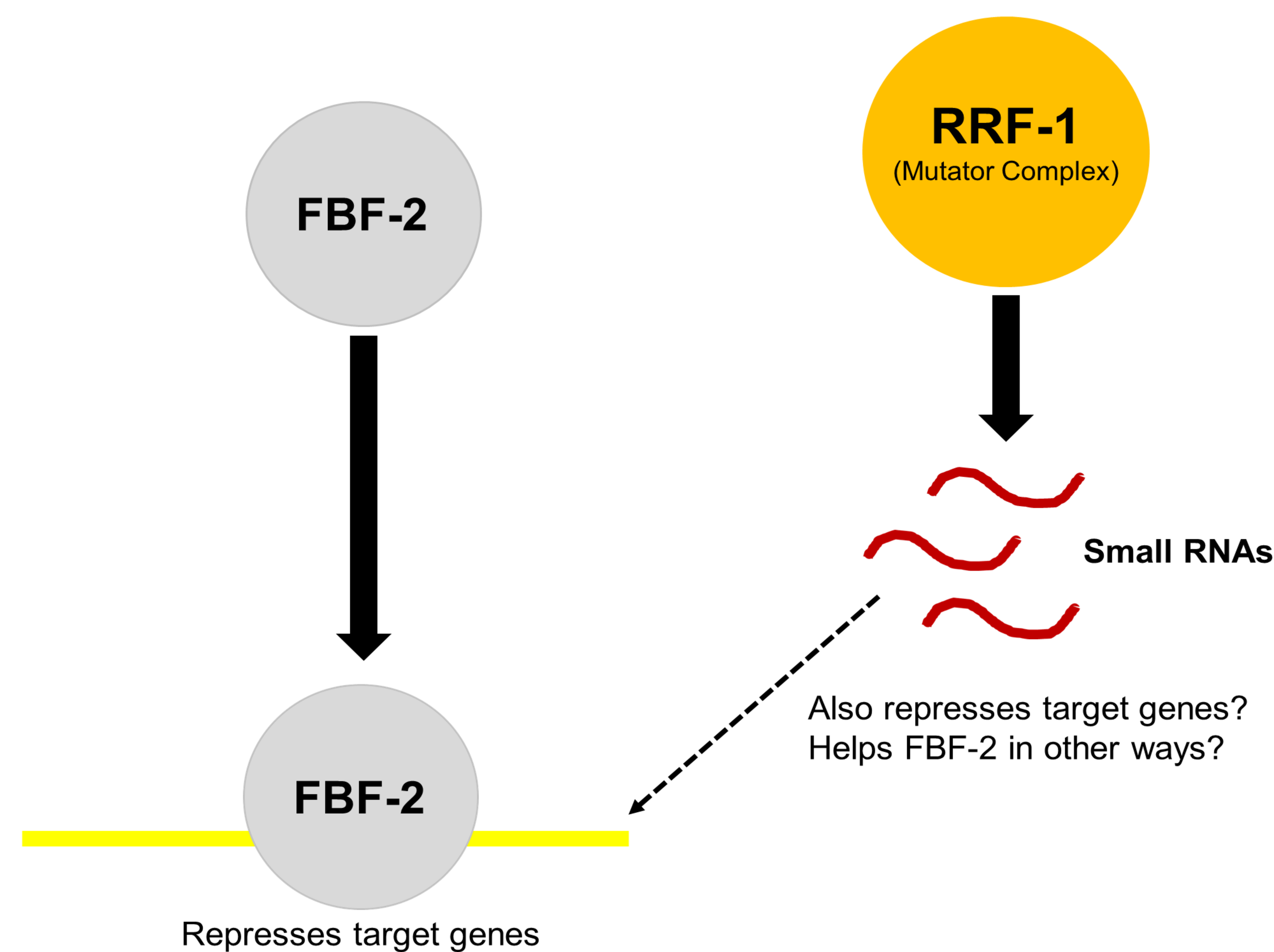
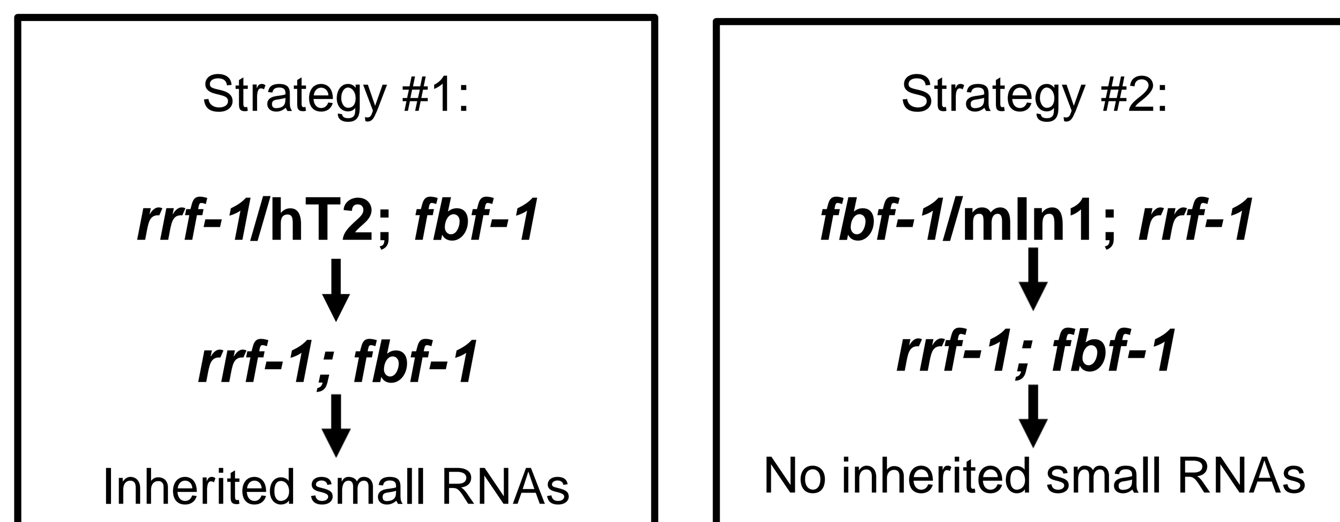


Figure 1. Model of FBF-2 and RRF-1 interaction.
FBF-2 represses the expression of target mRNAs in stem cells. RRF-1, an RNA polymerase, generates small regulatory RNAs. This model illustrates the potential interactions between RRF-1 and FBF-2.

2. Research Question

- Question: Does the rate at which *rrf-1* and *fbf-1* mutant *C. elegans* become sterile at 24° C change based on how the mutant is produced?

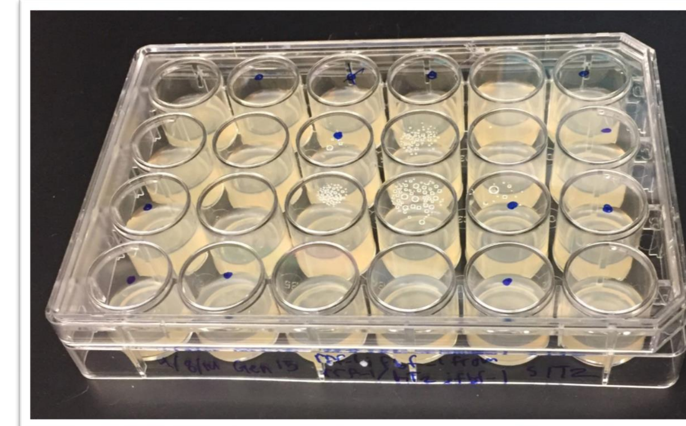


3. Methods

- Model organism: *C. elegans*
  - Nematode that has a short lifespan, is easy to maintain, and many of its proteins have mammalian orthologs.

1) **Genetic cross:** At 20° C, pick 7 male worms from one strain and 2 hermaphroditic worms from a different strain. Transfer the worms to a media plate with a small amout of food. Repeat this for two more days.

2) **Incubate worms at 24° C**



24-well plate for worm incubation. Each worm is isolated into its own compartment.

3) **Score number of sterile worms**

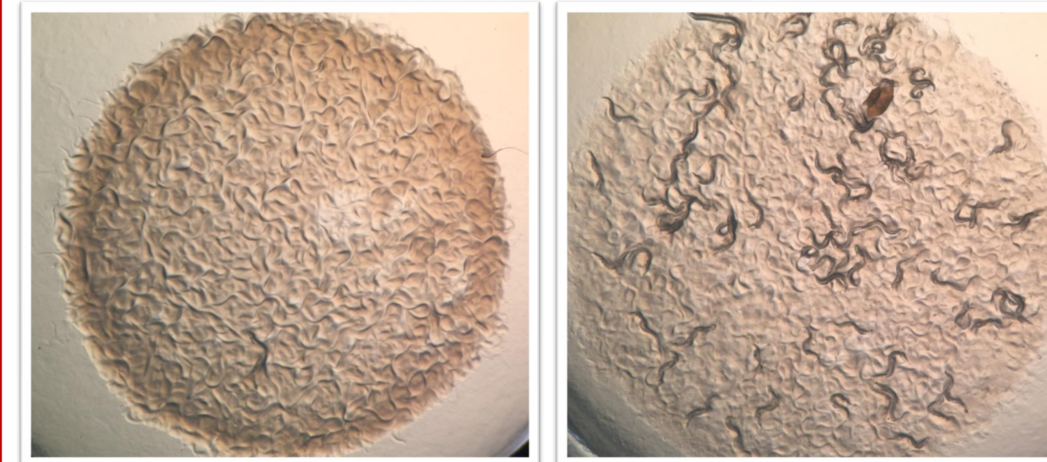


Image on left: well with sterile worm (single worm, no progeny). Image on right: well with fertile worm (numerous progeny observed).

4. Results

- *rrf-1; fbf-1* mutant progeny of *rrf-1/h*T2*; fbf-1 C. elegans* become sterile over time at 24° C as the amount of inherited RRF-1-generated small RNAs in the germline decreases.

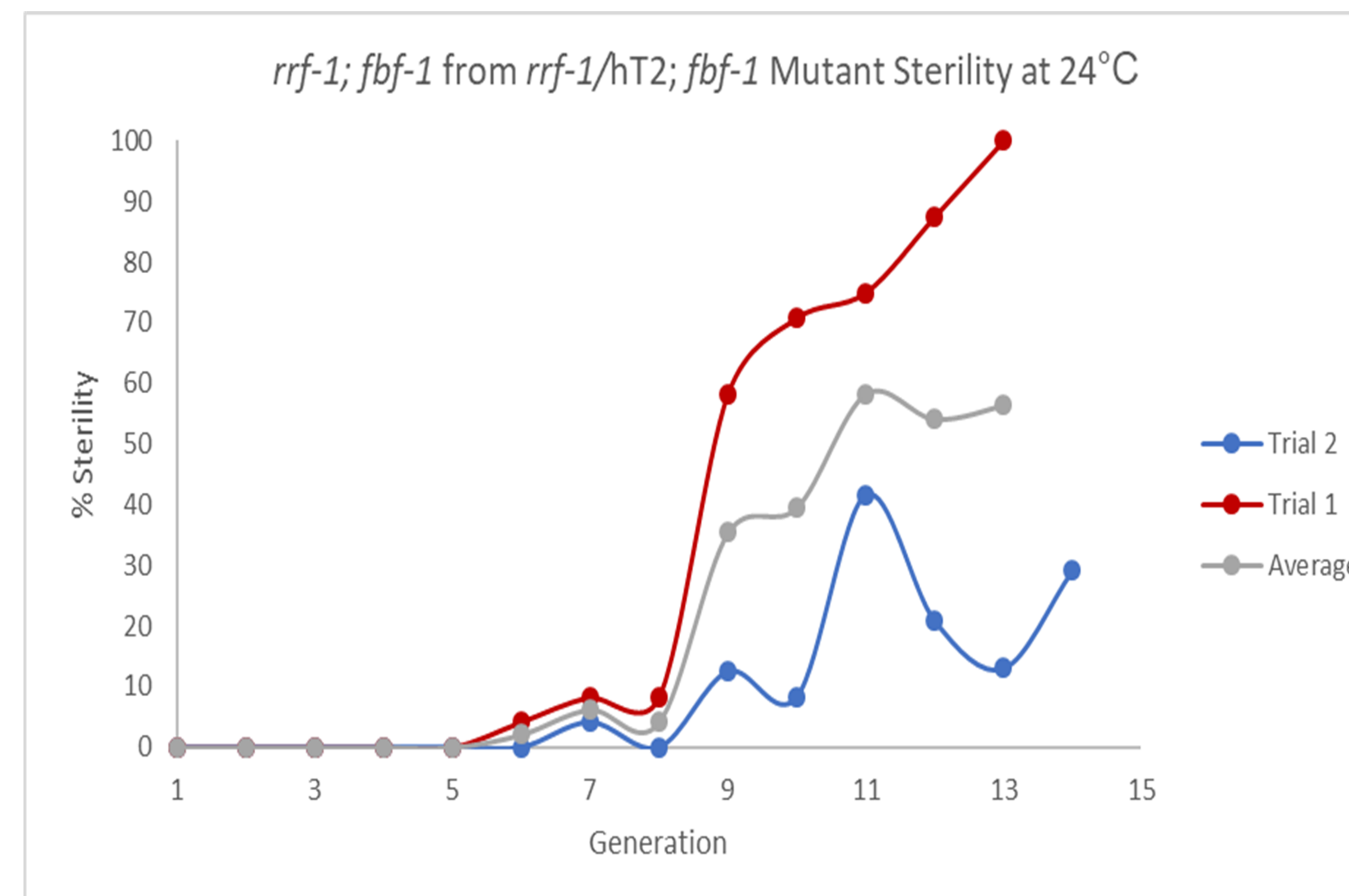


Figure 2. Graph of *rrf-1; fbf-1* from *rrf-1*/hT2; *fbf-1* mutant sterility per generation at 24° C
Over multiple generations, 24 *rrf-1; fbf-1* double mutant *C. elegans* were isolated into separate compartments of a multi-welled plate. They were incubated at 24° C for three days then scored for sterility. The percentage of sterile *C. elegans* was documented for each generation.

5. Discussion

- The results suggest that FBF-2 gradually loses its function over multiple generations after *rrf-1* is mutated. We can conclude this because the function of FBF-2 is isolated in the *rrf-1; fbf-1* double mutant strain.
- RRF-1 is part of an enzymatic mutator complex (mut-16) that localizes to P-granules (membrane-less ribonucleoprotein organelles) that are found in the germline (Phillips *et al.*, 2012). FBF-2 also localizes to P-granules.
- We were interested to see if the small RNAs produced by RRF-1 were promoting FBF-2-mediated silencing by targeting the same genes as FBF-2. We investigated this through a bioinformatic approach (Figure 3).
- After comparing thousands of genes, we found that there were only nine genes that overlapped, none of which are known to affect stem cell function (Figure 3).
- An alternative explanation of our data is that instead of working directly with FBF-2, RRF-1 is needed to silence expression of an unknown gene that represses or "turns off" FBF-2. Without RRF-1, there could be too much of this unknown gene and FBF-2 becomes inactivated, resulting in the *rrf-1; fbf-1* sterile phenotype that we see over time.

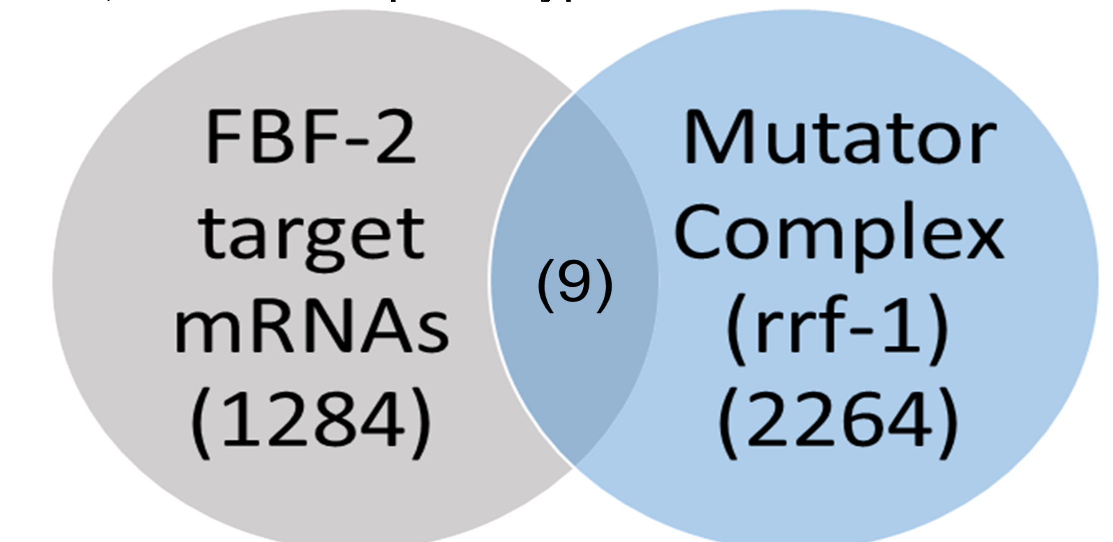


Figure 3. Diagram of genes that show significant loss of small RNAs when the mutator complex (mut-16) containing rrf-1 is disrupted and are FBF-2 target mRNAs.
A list of all known FBF-2 target mRNAs was compared to a list of genes that show a significant loss of small RNAs following disruption of mut-16 to identify genes that overlap. 1,284 FBF-2 targets were compared to 2,264 genes that had small RNAs affected by mut-16 disruption and nine genes were found in common.

6. Future Directions

- Complete strategy #2 (*rrf-1; fbf-1* double mutant generated from fbf-1/mIn1*; rrf-1* sterility at 24° C)
- Generate new *fbf-1/* mIn1*; rrf-1* strain with different *rrf-1* mutation (*rrf-1(ok589)*)
- RNA purification of small RNAs generated by RRF-1 and inject into *fbf-1/* mIn1*; rrf-1* strain of *C. elegans*

Literature Cited

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Acknowledgments

Thank you to Nick Day for the bioinformatic data on FBF-2 and RRF-1 and the members of the Voronina lab for their support. Funding provided by NIH ROIGMI09053.