Localizing *tra-2* mRNA in germ cells

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Abstract: *C. elegans* hermaphrodites are essentially female worms that have achieved self-fertility. Making sperm in an otherwise female body requires precise regulation of the feminizing gene, *tra-2*. While TRA-2 is ubiquitously expressed in the soma, its regulation is of special interest in the germ line where sperm are specified. There are two major mRNA isoforms of *tra-2*, *tra-2a* (4.7 kb) and *tra-2b* (1.8kb) (Okkema and Kimble 1991). The 1.8 kb transcript is specific to the hermaphrodite germline (Okkema and Kimble 1991, Kuwabara et al. 1998). The 4.7 kb transcript is predicted to be a transmembrane receptor for the male-specific protein HER-1 (Hamaoka et al. 2004). HER-1 represses *tra-2* activity to allow male cell fates in XO animals but is not expressed in XX hermaphrodites (Trent et al. 1991). Instead, available evidence suggests that hermaphrodites repress *tra-2* by the binding of a GLD-1/FOG-2 heterodimer to its 3' UTR (Jan et al. 1999, Clifford et al. 2000). While *tra-2* mRNA is abundant in the germline, epitope-tagged TRA-2 (TRA-2::HA) was undetectable in the presence of GLD-1 and FOG-2, and only slightly elevated in their absence (Hu et al. 2018). We are now investigating the regulation of *tra-2* at the mRNA level using single-molecule fluorescence in-situ hybridization (sm-FISH). We hypothesized that the 4.7 kb version of *tra-2* is not present in the hermaphrodite germline, as the 1.8 kb transcript contains all the domains necessary for interaction with both *fem-3* and *tra-1* (Mehra et al 1999, Lum et al 2000). The data suggest *tra-2a* is expressed in the germ line, but at lower levels than *tra-2b*. This indicates some TRA-2 membrane protein makes it to the ER, and perhaps even to the surface of hermaphrodite germ cells. Full-length TRA-2 could then be processed by TRA-3 as it is in the soma. *tra-2b* is consistently expressed in the rachis and oocytes of adult worms. We will further investigate *tra-2* regulation by using sm-FISH in a mutant that lacks GLD-1 binding sites on the 3'UTR (*tra-2(e2020)*). We expect this RNA to be de-regulated and potentially localize differently than wild type. We are also investigating the mechanism of FOG-2 action, with an emphasis on identification of additional protein-protein interactions.

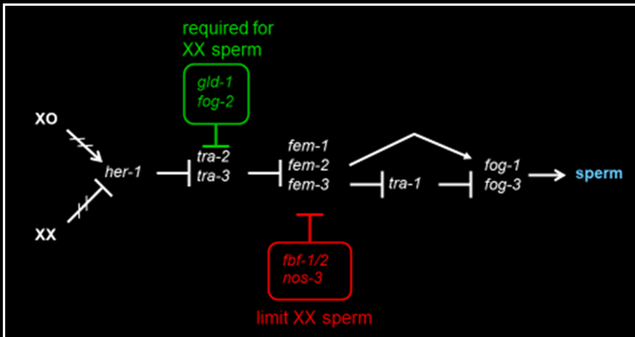


Figure 1. The *C. elegans* sex determination pathway. The ratio of X chromosomes to autosomes results in repression of *her-1* in hermaphrodites. Without *her-1*, *tra-2* feminizes the germline but is transiently repressed for sperm production. The presence of TRA-2 protein inhibits *fem-1*, *fem-2* and *fem-3*, allowing the transcription factor TRA-1 to specify female fate (for review, see Kuwabara and Kimble 1992).

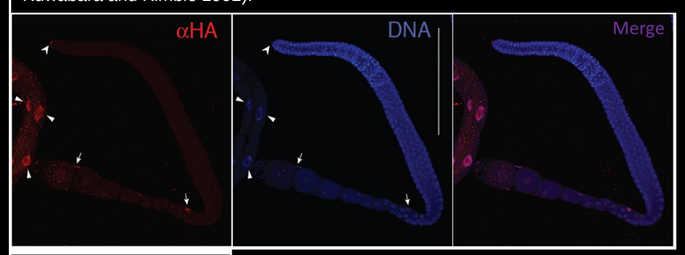


Figure 3. TRA-2::HA expression in the adult germ line. A confocal micrograph of an adult hermaphrodite gonad stained with αHA. TRA-2 protein is abundant in somatic cell nuclei (arrows) but is undetectable in the germ line (Hu et al. 2018).

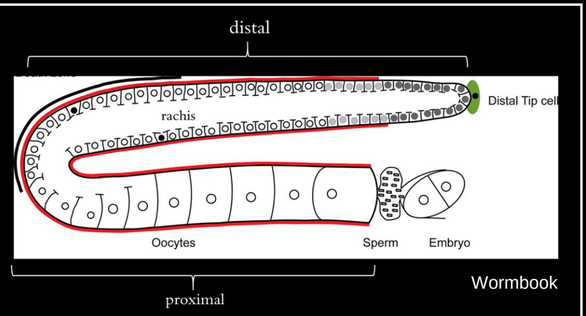


Figure 2. One arm of the *C. elegans* germ line. *C. elegans* hermaphrodites have two identical gonad arms. Each arm makes sperm in the L3 larval stage and switches to oogenesis. Germ cells are produced at the distal tip and are connected by a common cytoplasm called the rachis. Cells begin to differentiate as they move away from the distal tip. Oocytes move through the spermathecae, are fertilized and become embryos before being laid.

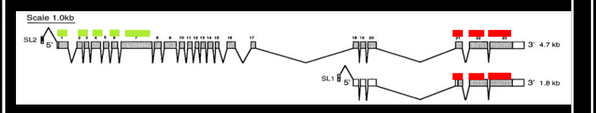


Figure 4. Differentiating *tra-2* germline isoforms. The short isoform of *tra-2*, *tra-2c* (1.8 kb), is oocyte-specific and contains the interaction domains for both FEM-3 and TRA-1 (Kuwabara et al. 1998). The 3' UTR of *tra-2a* and *tra-2c* are identical making it possible for both transcripts to be regulated by GLD-1 and FOG-2 (Kuwabara et al. 1998). It is possible that after oocyte production begins, this short transcript fulfills the roles of *tra-2* in the germline. We hypothesize that the 4.7 kb transcript is not present in the oogenic germ line. We decoupled *tra-2a* and *tra-2c* expression using sm-FISH (single-molecule fluorescent *in situ* hybridization) probes that can differentiate between transcripts, indicated by red and green bars using methods from Lee et al. 2016.

tra-2 expression in the absence of regulator binding sites

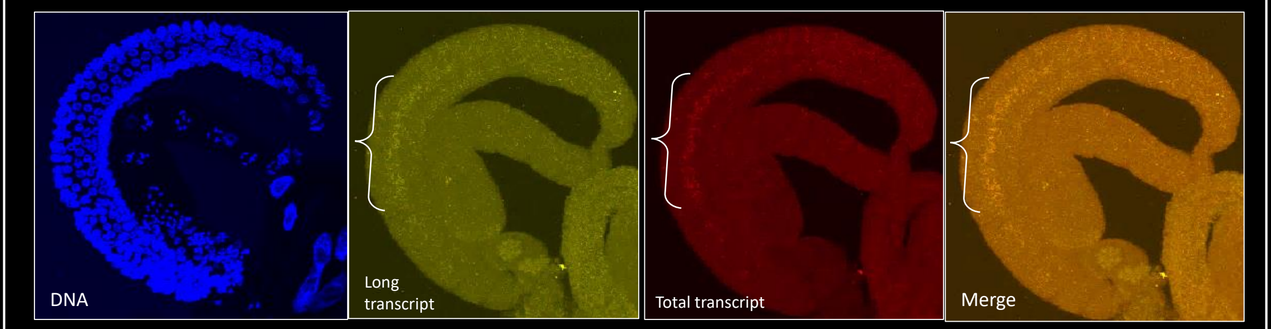
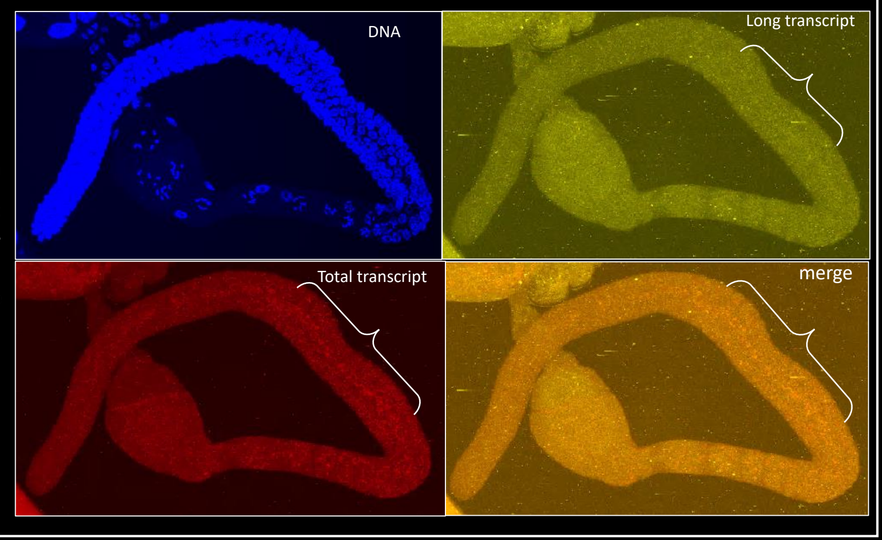


Figure 7. wild-type *tra-2* expression at 15C. Maximum projection confocal micrographs of dissected adult *C. elegans* germlines stained with sm-FISH probes for the long 4.7 kb transcript (green) and total *tra-2* transcript (red). Expression level and localization is qualitatively similar to 20C (see figure 5). Brackets highlight rachis expression.

Figure 8. *tra-2* expression in *tra-2(e2020gf)* at 15C. Maximum projection confocal micrographs of dissected adult germlines from mutant strain *tra-2(e2020). tra-2(e2020)* mutants lack all known GLD-1 (and FOG-2) binding sites on the *tra-2* 3' UTR. At 20C, both males and hermaphrodites are feminized. At 15C, the phenotype is slightly suppressed with males able to produce some sperm. *tra-2* mRNA is visibly present in the germline at 15C (shown here), but not at 20C (data not shown). At 15C, *tra-2* expression can be seen in the rachis (brackets). The data suggest that *tra-2* mRNA is partially regulated independent of the GLD-1/FOG-2 complex at the suppressive temperature (15C). Germline *tra-2* expression is very robust in *tra-2(e2020)* mutants at 15C, but animals are not as strongly feminized at 15C as at 20C where there is no visible *tra-2* expression. We hypothesize that at 20C, the very little *tra-2* mRNA present is completely de-regulated and therefore ultra-feminizing (see Goodwin et al. 1993 for northern blot).



Is 5Kb *tra-2* transcript present in the germ line?

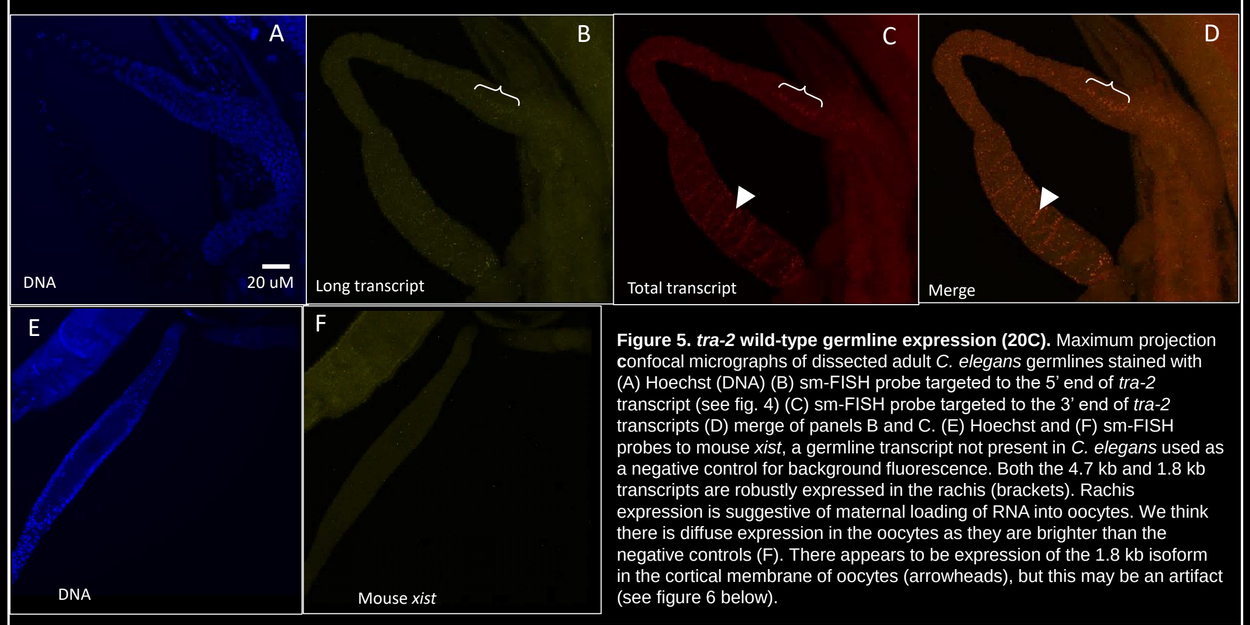


Figure 5. *tra-2* wild-type germline expression (20C). Maximum projection confocal micrographs of dissected adult *C. elegans* germlines stained with (A) Hoechst (DNA) (B) sm-FISH probe targeted to the 5' end of *tra-2* transcript (see fig. 4) (C) sm-FISH probe targeted to the 3' end of *tra-2* transcripts (D) merge of panels B and C. (E) Hoechst and (F) sm-FISH probes to mouse *xist*, a germline transcript not present in *C. elegans* used as a negative control for background fluorescence. Both the 4.7 kb and 1.8 kb transcripts are robustly expressed in the rachis (brackets). Rachis expression is suggestive of maternal loading of RNA into oocytes. We think there is diffuse expression in the oocytes as they are brighter than the negative controls (F). There appears to be expression of the 1.8 kb isoform in the cortical membrane of oocytes (arrowheads), but this may be an artifact (see figure 6 below).

FOG-2 protein-protein interaction studies

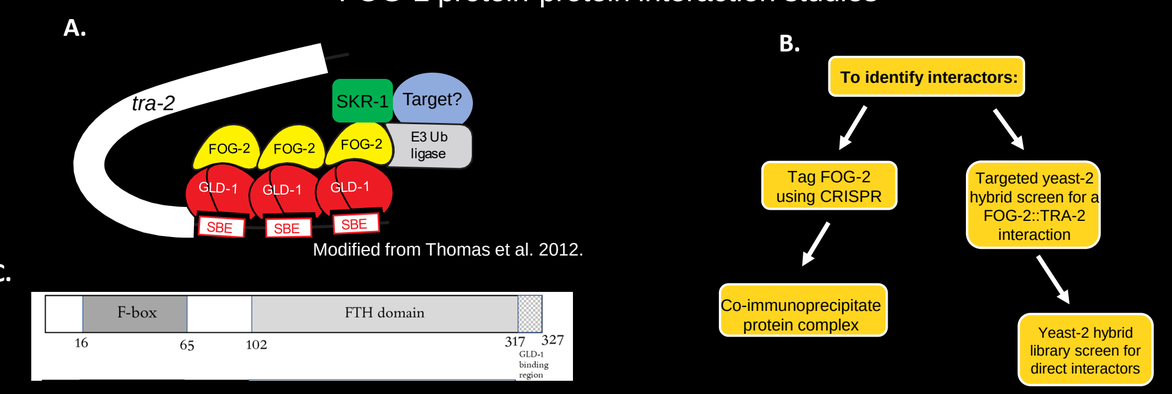


Figure 9. FOG-2 protein-protein interactions. (A) A representation of GLD-1 (red) and its binding partner FOG-2 (yellow) bound to the 3' UTR of *tra-2* mRNA (white) on 3 tandem STAR binding elements (SBE) (Clifford et al. 2000, Jan et al. 1999). This interaction is necessary for hermaphrodite spermatogenesis. FOG-2 is *C. elegans*-specific and necessary for hermaphrodite, but not male, sperm production (Schedl and Kimble 1988, Clifford et al. 2000). The mechanism of FOG-2 action is unknown but identifying interactors could reveal a target of the FOG-2 F-box. FOG-2 is known to interact with SKR-1 (green), a component of the ubiquitin ligase pathway (Nayak et al. 2005). (B) A schematic of planned FOG-2 interaction studies. (C) If the F-box and its interaction with SKR-1 is necessary for XX spermatogenesis, mutations in important residues, like *fog-2* null alleles, should render a feminization-of-germline (Fog) phenotype. We will alter amino acids 44-63 of the F-box domain using CRISPR to disrupt its potential interaction with the ubiquitin ligase pathway. The schematic of FOG-2 above shows the three domains: the F-box, *ftr-1* homology domain (FTH) and the GLD-1 binding region. Numbers below indicate amino acids.

Cortical oocyte staining is likely an artifact

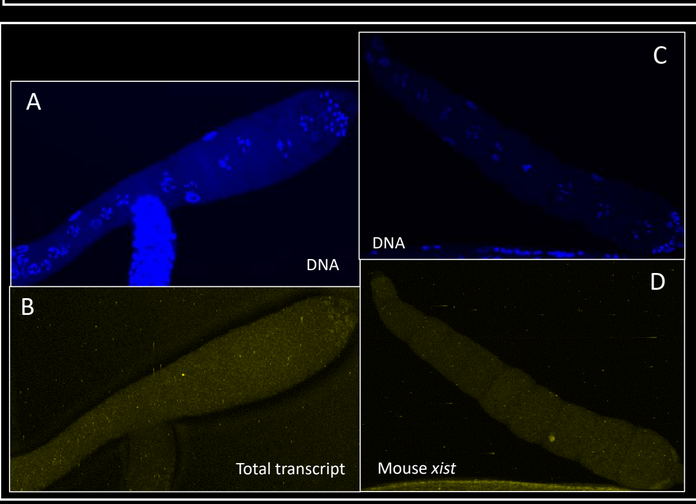


Figure 6. Total *tra-2* transcript in proximal oocytes stained with alternative probe set. Maximum projection confocal micrographs of proximal oocytes stained with A) Hoechst (DNA) B) green sm-FISH probe set targeted to the 3' end of *tra-2* transcripts C) Hoescht and D) sm-FISH probes to mouse *xist*. Cortical oocyte staining was only visible in gonads stained with red sm-FISH probe targeted to the 3' end of *tra-2* transcripts (figure 5 C and D). To test for specificity of this signal, we stained gonads with identical probes but in the opposite color (green). No cortical oocyte staining was seen (N=5). Proximal oocytes stained for total *tra-2* transcript (B) were consistently brighter than mouse *xist* negative controls (D) leading to the hypothesis that *tra-2* mRNA in oocytes is diffuse throughout the cytoplasm. The probes comprising the red and green versions of the *tra-2* 3' probe sets are identical but were imaged at different intensities because green probe sets have higher background fluorescence in these experiments.

References

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Acknowledgments

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