Characterization of a potential gene interaction between *spr-5, met-2*, and *mep-1* in determining germline soma distinctions in *C. elegans*

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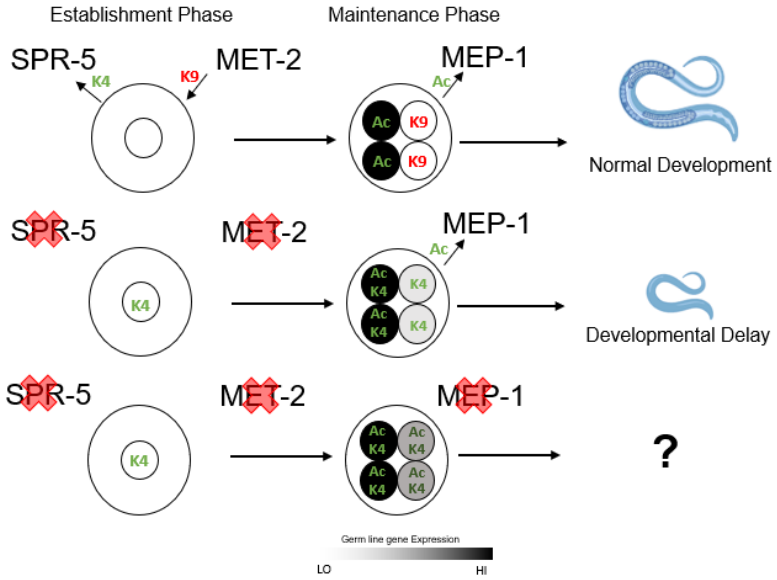
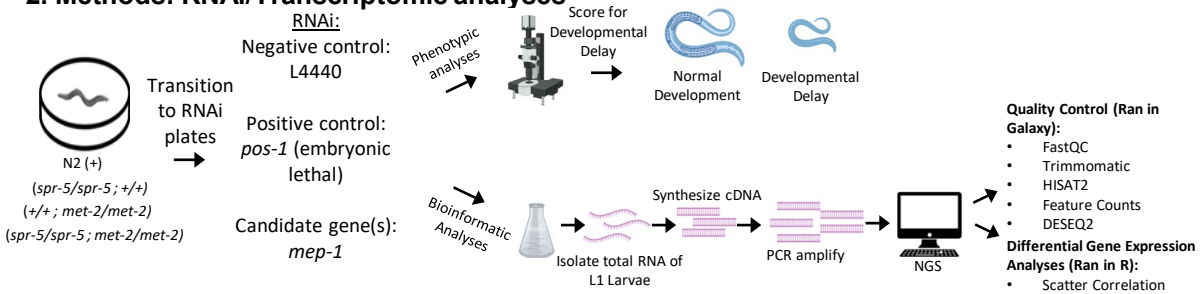
Abstract. In *C. elegans*, epigenetic modifier proteins aid in the activation and repression of gene expression that is required to distinguish germline from soma. Two of these epigenetic modifiers, SPR-5 and MET-2 work synergistically to establish a totipotent ground state by shutting down the precocious transcription of germline specific genes inherited from the gametes. Previous studies have shown that unlike *spr-5* or *met-2* single mutants, *spr-5; met-2* double mutants produce progeny that experience a severe developmental delay in stage 1 larvae (i.e., organisms without a developed germline). Transcriptomic analysis of these animals indicates that the delay is largely due to the inappropriate expression of genes required for meiosis. Here we explore the possibility that the early actions of SPR-5 and MET-2 are reinforced in the embryo (until a germline is formed) by coordinating with other transcriptional regulators. One possible collaborator is MEP-1, a component of the NuRD like epigenetic deacetylase complex, MEC. Similar to *spr-5; met-2* double mutants, *mep-1* mutants fail to suppress germline genes as indicated by the misexpression of PGL-1 and GLH-3 proteins in the soma of L1 larvae. Here we test a potential interaction between these two pathways by subjecting *spr-5 and met-2* mutants (both single and double) to *mep*-1 RNAi. *mep-1* knockdown when combined with any *spr-5/met-2* mutant background, results in an exacerbated developmental delay phenotype. The "triple" mutant is most severe, displaying a full L1 arrest. To fully understand the molecular basis of this synergy, we scaled up *mep-1* RNAi to generate sufficient quantities of L1 isolates to perform an RNA-seq based transcriptomic analysis. Data has been analyzed for overlapping gene expression patterns between *spr-5, met-2, and mep-1*, which, if present, would be consistent with a functional gene interaction between these pathways. Upon analyses, the data indicates that MEP-1 acts on the same gene targets as SPR-5 and MET-2 in the zygotic animal. Collectively, these basic findings could help elucidate the functional mechanisms of human orthologues that have been implicated in neurological diseases.

1. Germline/Somatic cell fates are established through epigenetic histone modifying enzymes

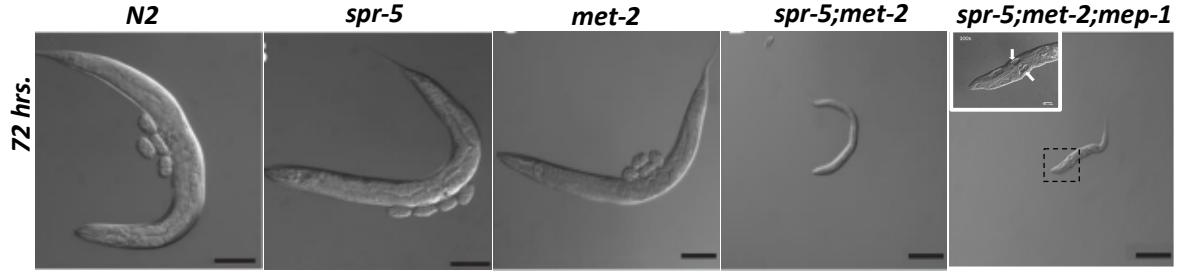


HYPOTHESIS: *mep-1 collaborates with* the maternally deposited chromatin modifying genes *spr-5* and *met-2* to prevent germline gene expression in the soma.

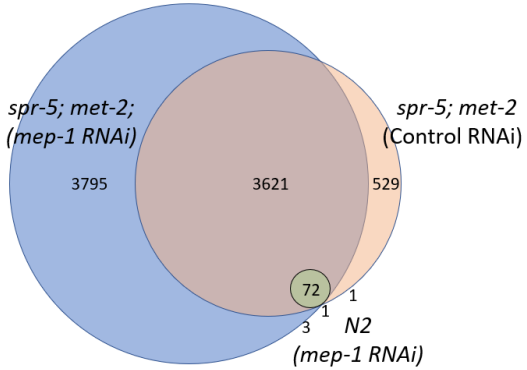
2. Methods: RNAi/Transcriptomic analyses



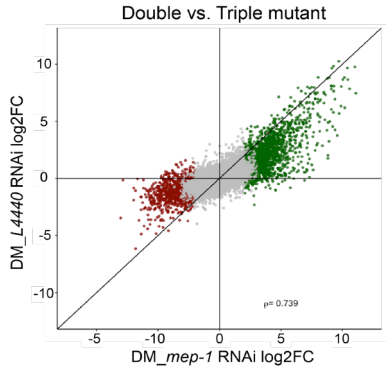
3. Loss of *mep-1* in *spr-5; met-2* progeny, exacerbates developmental delay phenotype



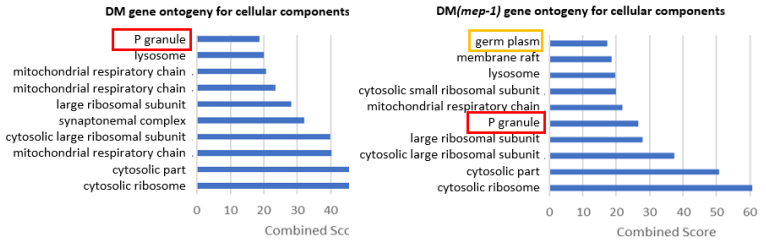
4. DM and DM(*mep-1*) DEGs overlap significantly



5. DM DEGs are amplified in DM(mep-1)



6. Expression of germline genes is enriched in DM*(mep-1)*



7. Discussion

- *mep-1* works with *spr-5* and *met-2* to suppress inappropriate germline gene expression in the soma
  - Developmental delay in DM(*mep-1*) is more severe than in DM.
  - Gene misexpression in DM(*mep-1*) is more amplified than in DM at same gene loci.
  - DM(*mep-1*) DEGs are enriched in germline targets.
- Future Studies:
  - MEP-1 ChIP-seq comparisons of N2 vs DM.
  - ATAC-seq comparisons of DM vs DM(*mep-1*)

References/Acknowledgments:

- Kerr, S.C., Ruppersburg, C.C., Francis, J.W., Katz, D.J., 2014. SPR-5 and MET-2 function cooperatively to reestablish an epigenetic ground state during passage through the germ line. Proc. Natl. Acad. Sci. U.S.A. 111, 9509–9514. doi:10.1073/pnas.1321843111
- Unhavaithaya, Y., Shin, T.H., Miliaras, N., Lee, J., Oyama, T., Mello, C.C., 2002. MEP-1 and a homolog of the NURD complex component Mi-2 act together to maintain germline soma distinctions in C. elegans. Cell 111, 991–1002