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The Role of Chromatin Modification in Germ Cell Specification and Development

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Chromatin Modifications and Fertility

Chromatin modifications are modifications of proteins called histones that regulate the compaction of DNA so it can fit in the nucleus of the cell. These modifications include methylation or acetylation of histones, and can result in activation or repression of transcription of the DNA, which is essential for the cell to synthesize proteins and pass on genetic information. Some chromatin modifications have been linked to fertility and specification of reproductive cells called germ cells.

Objectives

- Study chromatin modification H3 lysine-9 trimethylation (H3K9me3) that methylates histones causing transcriptional repression and has not been previously identified in germ cells
- Identify the enzyme that regulates this modification by testing various mutant strains
- Determine whether the loss of H3K9me3 is linked with loss of fertility

Hypothesis

- The loss or decline of H3K9me3 in germ cells will affect reproductive cells specification and fertility

Methods

- *C. elegans*, a eukaryotic nematode, was used as the model organism
- *C. elegans* shares similar reproductive regulation mechanisms as humans do, so results from this study can contribute to a better understanding of the mechanism in the development of germ cells in humans
- Used mutant strains with different methyltransferase mutations, some linked to sterility
- Examined presence of H3K9me3 in germ cells compared to somatic cells by using indirect immunofluorescence
- Compared the loss of H3K9me3 in germ cells with mutants known to be sterile to determine correlation

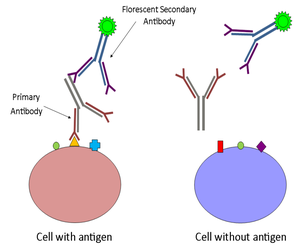
Immunofluorescence

- specific antibodies used to stain and identify the germ cells and the H3K9me3 methylation

1. Primary antibody binds to antigen

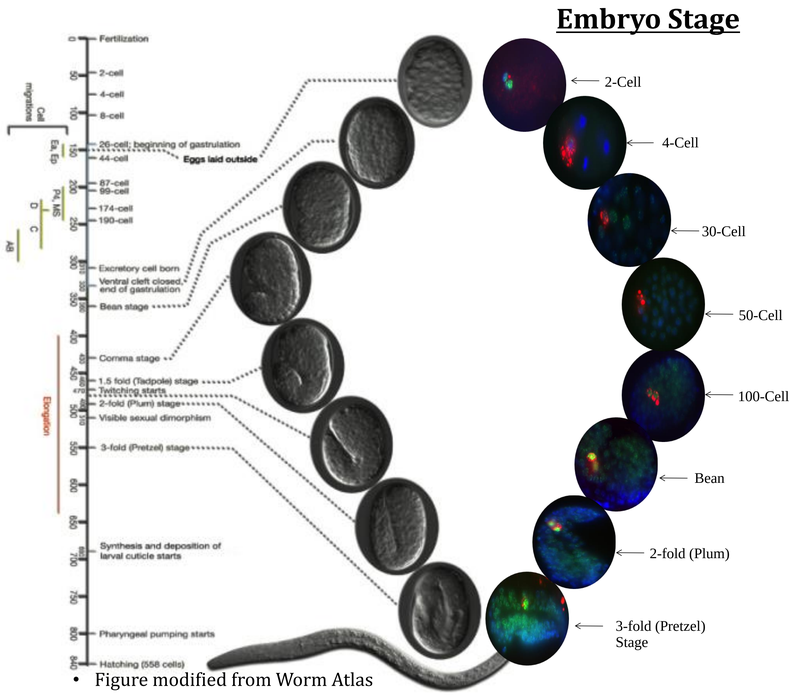
2. Secondary antibody with fluorescence binds to primary antibody, "highlighting" it

Figure 1: Mechanism of Immunofluorescence Staining



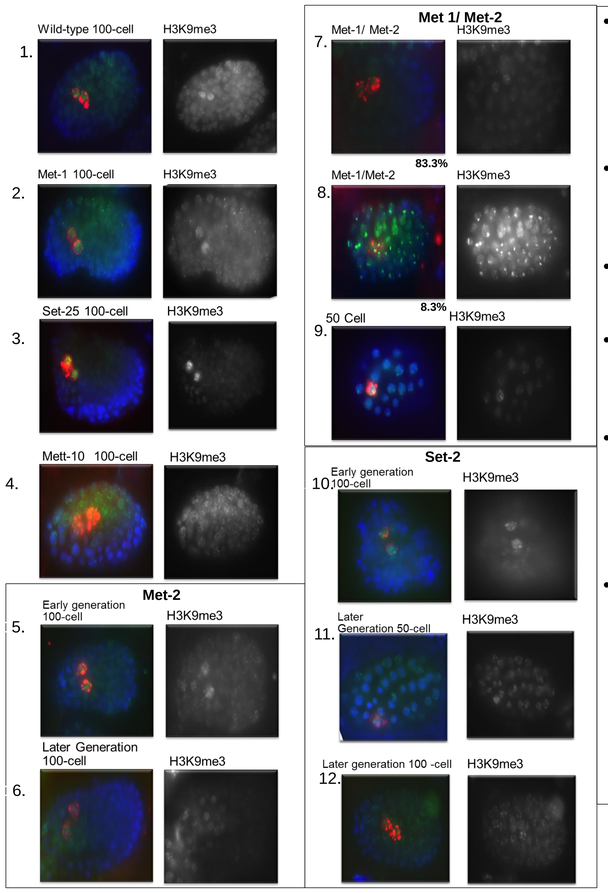
- Figure from University of Illinois, College of Engineering

Figure 2*: C. Elegans* Germline and Histone Trimethylation of H3K9



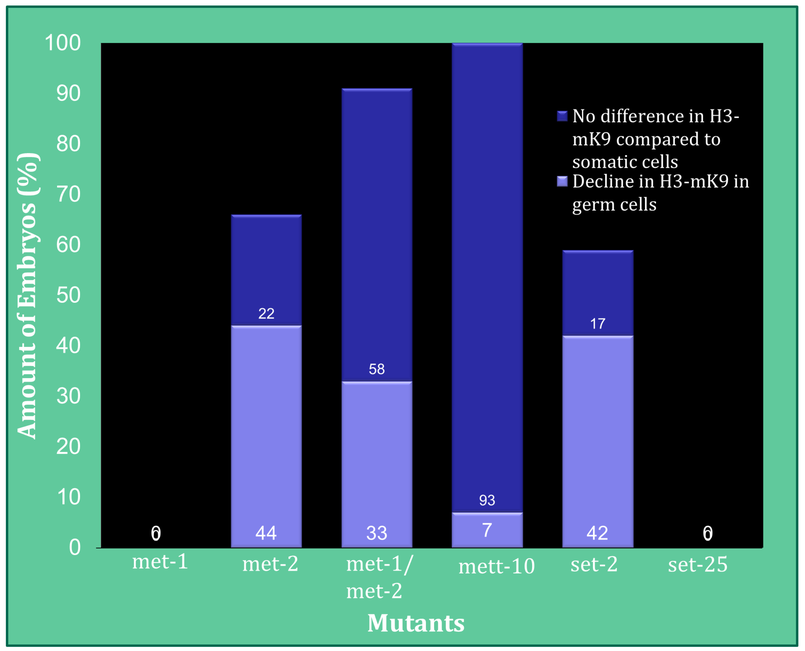
- Figure modified from Worm Atlas

Figure 3: Mutants in Methyltransferases and Presence of H3K9 Trimethylation



- *C. elegan's* embryos stained with anti-H3K9me3 (green), P granules (**red**) highlighting germ cells, and DNA (blue).
- **Panels 1-3**: Normal H3K9me3 in wild-type, met-1, and set-25 embryos
- **Panel 4:** Loss of excess H3K9me3 in germ cells in mett-10
- **Panels 5 & 6**: Staining of met-2 mutant in early versus late generations of worm
- **Panels 7-9**: Different patterns observed of H3mK9me3 in 100-cell in met-1/met-2 (Panels 7 & 8) and in 50-cell (panel 9)
- **Panels 10-12:** Comparison of staining of early versus later generation of set-2 in 100-cell and difference between 50 and 100-cell in later generation

Figure 4: Percent of Embryos with Loss of H3-K9 Methylation in Germ Cells



- Met-2 and Set-2 data include embryos from early and later generations

Correlation of Loss of H3K9 Trimethylation with Sterility

Mutant	Methylation disrupted (according to literature)	Excess of H3K9me3 in Germ Cells compared to somatic cells	Sterile?
Met-1	H3K36me3	+	No
Met-2	H3K9me2	-	Yes, overtime
Met-1/Met-2	H3K36me3 & H3K9me2	-	Yes
Mett-10	unknown	-	Partial
Set-2	H3K4me3	-	Yes, overtime
Set-25	H3K9me3	+	No

Results

- Mutants tested so far reveal a strong correlation between loss of excess H3K9me3 in germ cells and increasing sterility
- In Set-2 and Met-2 mutants, H3K9me3 decreases in germ cells over time, as the worms become more sterile
- Set 2 and Met-1/ Met-2 mutants appear to not lose H3K9me3 until the later 100 cell stage when 2 germ cells are present

Future Research

- Continue to test other mutants known to be sterile
- Investigate why Set-25 (supposed to be disrupted in H3K9me3) had a phenotype like the wild-type
- Analyze what stage in embryo development H3K9me3 is lost and the regulator of it