Characterization of Histone Modification Throughout *C. elegans* Embryogenesis

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Introduction

Mechanisms of embryonic development and the transition from cellular plasticity to differentiated cell types have long been an area of great interest. One method to characterize this progression is by studying changes in nuclear organization, controlled in part by specific modification of histone tails (Fig. 1). Different modifications may either promote or repress transcription; the overall consequences of histone modification include chromatin remodeling and gene expression (Fig. 2). In this project we analyzed a number of key modifications including H3K27trimethylation, H3K9me3, H4K16 acetylation, and the modifying protein poly(ADP-ribose) or PAR using immunohistochemistry in *Caenorhabditis elegans.* We then characterized the localization and concentration of these modifications throughout the developmental stages (2E-8E) specifically looking for changes at the transition from cellular plasticity to differentiation.

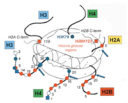


Figure 1. Histone modification sites *(1).*

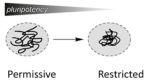
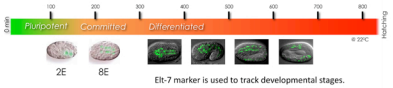


Figure 2. A chromatin based model for the loss of cell plasticity.

C. elegans Development

- Somatic blastomeres are characterized through their Endodermal (E) stages (Fig. 3).
- Before 8E, embryonic cells are developmentally plastic and can take on a variety of cell types.
- After 8E (100 cells) cells become committed to a specific cell fate.



Elt-7 marker is used to track developmental stages.

Figure 3. Stages of *C. elegans* embryonic development.

Objectives

- To document the changes in the histone modifications over time.
- To test antibody specificity in *C. elegans*.

Methods

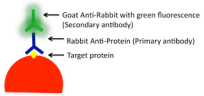


Figure 4. Methodology of Immunohistochemistry.

- We used immunohistochemistry to stain histone modifications in embryos (Fig. 4).
- Embryos were obtained from bleached adults and from the worm plates and fixed using paraformaldehyde.
- Commercially available primary antibodies included H3K9me3, H3K27me3, and H4K16acetylation. We also used POPO (Invitrogen/ Molecular probes) to stain for nucleic acid.
- Secondary antibodies were tagged with green and red fluorescent dyes.
- Fluorescence microscopy as well as confocal microscopy were used to image stained embryos.

Results

Changes of Histone Marks during Embryogenesis

❑ **H3K27me3**

- H3K27me3 is a repressive mark that is associated with histone compaction and restriction of gene transcription.
- H3K27me3 stains show ubiquitous expression throughout the embryonic development (Fig. 5).

❑ **H4K16acetylation**

- H4K16acetylation is an activating mark that is associated with histone de-compaction and activation of gene transcription.
- H4K16acetylation stains show a decreased number of histone stained nuclei in later development (Fig. 5).
- In the H4K16ac stain, older embryos usually show a few bright nuclei in specific cells.

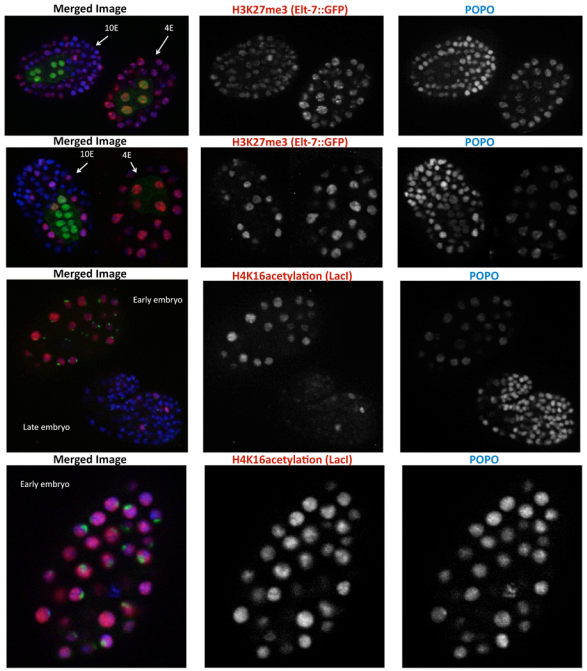


Figure 5. Expression of H3K27me3 and H4K16ac in early and late embryos.

Histone Marks and Chromatin Compaction

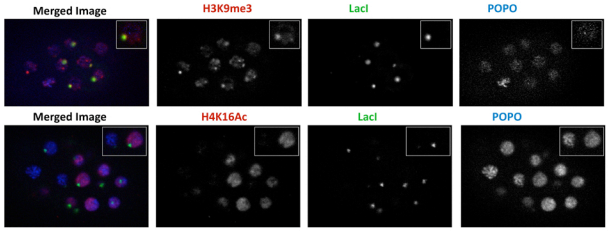


Figure 6. Colocalization of LacI and repressive histone mark.

- LacI::CFP arrays form a pseudo-chromosome that can be easily traced using immunohistochemistry and is used to assess chromatin compaction (Fig. 7).
- The shape of the pseudo-chromosome is indicative of its state.
- Floret = open/active gene transcription
- Elipsoid = compact/repressed gene transcription
- LacI marked pseudo-chromosomes (elipsoids) are enriched with H3K9me3, a repressive mark (Fig. 6).
- H4K16ac stain is excluded from elipsoids.

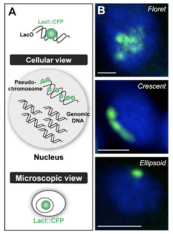


Figure 7. LacI ::CFP in Embryonic Cells (2).

Absence of PAR in Embryos

- PAR acts as a histone acceptor and enables transcription activation (3).
- PAR staining is only seen in the germline of adult worms and ooctyes (Fig. 8).
- This modification is absent in developing embryos.

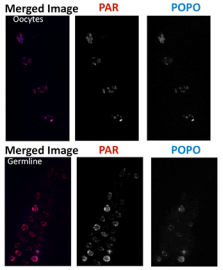


Figure 8. Presence of PAR in oocytes and germline

Conclusions

Table 1. Histone modification in embryos

Histone Marker	Type	Present In
H3K9me3	Repressive	All Embryonic Stages
H3K27me3	Repressive	All Embryonic Stages
H4K16 Acetylation	Activating	Younger stages Fewer cells at 12E
Poly(ADP-ribose)	Activating	Germline only

- H3K27me3 shows consistent expression throughout the developmental stages of embryogenesis and is brighter in mitotic nuclei.
- H4K16acetylation is exclusively seen in interphase nuclei and is generally more prominent in the younger stages of development.
- H4K16ac shows a decrease in expression at around the 12E stage.
- H3K9me3 is associated with compact and repressed chromatin.
- Poly (ADP-ribose) expression is seen in oocytes and germline nuclei.

Future Directions

- Continue imaging H4K16Ac to pinpoint exact time of change in expression.
- Quantify data to better identify trends.
- Determine the role Poly (ADP-ribose) in germline nuclei.
- Identify the mechanism of PAR removal from embryonic nuclei.
- Identify enzymes that modify specific histone tail residues.
- Determine how histone marks affect gene expression.
- Analyze other histone modifications.

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References

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