Genome-wide patterns of histone H2A monoubiquitylation and effects on *C. elegans* developmental timing

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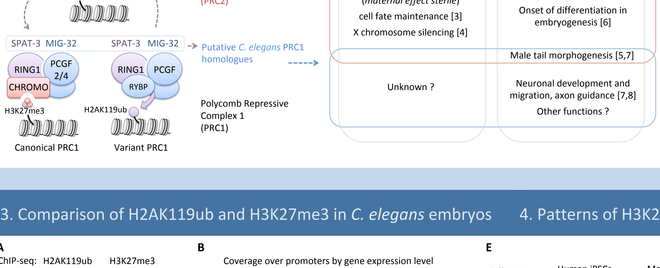
1. Histone modifications by Polycomb Repressive Complexes

Background:
Histone H2A mono-ubiquitylation is a post-translational modification associated with the regulation of gene expression and development. In *Drosophila* and mammals, canonical and variant/non-canonical forms of Polycomb Repressive Complex 1 (PRC1) mediate histone H2A mono-ubiquitylation (H2AK119ub) and chromatin compaction [reviewed in 1]. The PRC1 complexes are believed to work cooperatively with PRC2-mediated histone H3 lysine 27 trimethylation (H3K27me3) to repress gene expression. However, the distribution of H2A ubiquitylation across the genome and its role in developmental gene regulation are not fully understood.



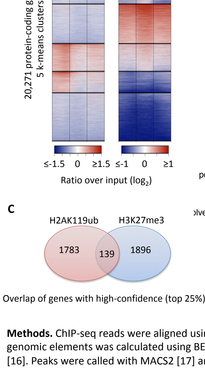
2. Questions & Approach

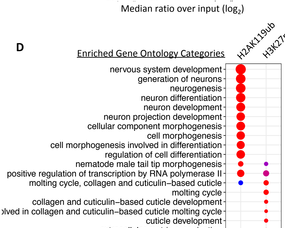
Rationale:
The roles of Polycomb Repressive Complexes in silencing developmental regulators in pluripotent cells have been well-established [reviewed in 9]. However, PRC complexes may function independently in other contexts, and variant PRC1 may have dynamic effects on gene expression [10,11]. The phenotypes of PRC2 and potential (v)PRC1 homologues in *C. elegans* suggest that these complexes may play partially distinct roles.

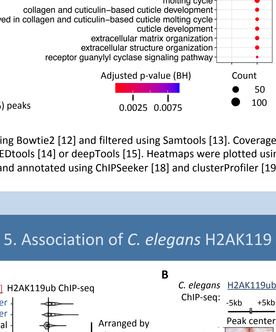
Questions:
- What is the relationship between H2AK119 mono-ubiquitylation and H3K27me3 in *C. elegans*?
- Is H2AK119ub associated with repressed genes?
- Do genomic targets of H2AK119ub show functional enrichment?
- What are the characteristics of H2AK119ub-marked genomic loci?

Approach:
- We performed ChIP-seq in *C. elegans* embryos and compared genomic patterns of H2AK119ub to other modifications and chromatin states
- We investigated phenotypes of H2A ubiquitylation-deficient mutants using synchronized development assays and ChIP

3. Comparison of H2AK119ub and H3K27me3 in *C. elegans* embryos

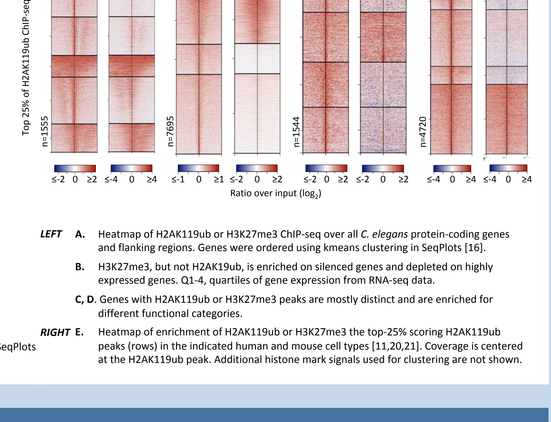






Methods. ChIP-seq reads were aligned using Bowtie2 [12] and filtered using Samtools [13]. Coverage of genomic elements was calculated using BEDtools [14] or deepTools [15]. Heatmaps were plotted using SeqPlots [16]. Peaks were called with MACS2 [17] and annotated using ChIPSeeker [18] and clusterProfiler [19].

4. Patterns of H3K27me3 at H2AK119ub peaks in mammalian cells



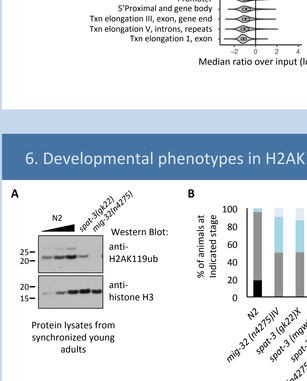
LEFT **A.** Heatmap of H2AK119ub or H3K27me3 ChIP-seq over all *C. elegans* protein-coding genes and flanking regions. Genes were ordered using kmeans clustering in SeqPlots [16].

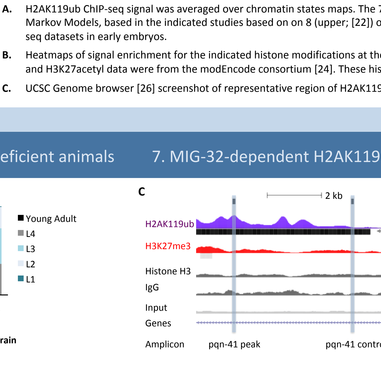
B. H3K27me3, but not H2AK119ub, is enriched on silenced genes and depleted on highly expressed genes. Q1-4, quartiles of gene expression from RNA-seq data.

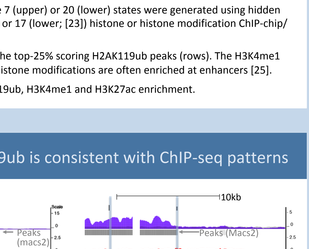
C, D. Genes with H2AK119ub or H3K27me3 peaks are mostly distinct and are enriched for different functional categories.

RIGHT **E.** Heatmap of enrichment of H2AK119ub or H3K27me3 the top-25% scoring H2AK119ub peaks (rows) in the indicated human and mouse cell types [11,20,21]. Coverage is centered at the H2AK119ub peak. Additional histone mark signals used for clustering are not shown.

5. Association of *C. elegans* H2AK119 mono-ubiquitylation with enhancer-like domains







A. H2AK119ub ChIP-seq signal was averaged over chromatin states maps. The 7 (upper) or 20 (lower) states were generated using hidden Markov Models, based in the indicated studies based on on 8 (upper; [22]) or 17 (lower; [23]) histone or histone modification ChIP-chip/seq datasets in early embryos.

B. Heatmaps of signal enrichment for the indicated histone modifications at the top-25% scoring H2AK119ub peaks (rows). The H3K4me1 and H3K27acetyl data were from the modEncode consortium [24]. These histone modifications are often enriched at enhancers [25].

C. UCSC Genome browser [26] screenshot of representative region of H2AK119ub, H3K4me1 and H3K27ac enrichment.

6. Developmental phenotypes in H2AK119ub-deficient animals

A. Western blots of bulk H2AK119 ubiquitylation levels in wild-type (N2) and strains with deletions in *mig-32* or *spat-3*, putative homologues of PRC1 components PCGF/Bmi1 and RING1, respectively [7,8].

B. Quantification of animal stage during synchronous development. Animals (380-794 per genotype) were scored 45 hours after release from L1 diapause. The *spat-3(mgw14)* allele is a point mutation [8]

C. UCSC genome browser [26] tracks of ChIP-seq coverage for two representative loci in wild-type embryos. Positions of MACS2-predicted peaks are shown below the coverage tracks and coloured by score (darker, higher confidence). Positions of PCR amplicons for ChIP-qPCR are indicated.

7. MIG-32-dependent H2AK119ub is consistent with ChIP-seq patterns

D. Directed ChIP-qPCR assays in wild-type and *mig-32(n4275)* mutant embryos for amplicaons indicated in panel **A**. Control amplicons are regions of low enrichment in the ChIP-seq.

8. Summary

Conclusions:
- In *C. elegans* embryos, H2AK119ub and H3K27me3 are *not* generally enriched at the same genomic locations
- In mammalian cells, there may be a trend towards more co-enrichment of H2AK119ub and H3K27me3 in pluripotent versus differentiated cell types
- Histone H2AK119ub may co-localize with enhancer-associated chromatin modifications. Notably, RING1B also localizes to enhancers in cancer cells [20]
- Enrichment of H2AK119ub at genes involved in neuronal development is consistent with known phenotypes of *mig-32* and *spat-3* mutants [7,8]
- *mig-32* and *spat-3* mutant animals are deficient in H2AK119ub [7,8 and this work] and show developmental abnormalities

Future Directions:
- What is the relationship between H2AK119ub and H2A variants?
- What is the functional significance of H2AK119ub localization at enhancer-like loci (e.g. a bivalent/poised state)?
- Are the developmental phenotypes of *mig-32* and *spat-3* mutant animals dependent on H2AK119ub?
- Is loss of *mig-32*/*spat-3* sufficient for misregulation of the H2AK119ub-marked loci?
- How does loss of H2AK119ub affect H3K27me3 levels?
- Are MIG-32 and SPAT-3 part of canonical or variant PRC1-like complexes?

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