

Histone H1 and heterochromatin protein 1 (HP1) regulate specific gene expression and not global transcription

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The highly conserved Hox transcription factors define positional identity along the anterior-posterior body axis during development. Inappropriate expression of Hox genes causes homeotic transformation, which leads to abnormal development of a specific region or segment. *C. elegans* offers an excellent model for studying factors required for the establishment of the spatially-restricted expression of Hox genes. We have recently identified chromatin factors, including a linker histone (H1) variant, HIS-24 and heterochromatin protein 1 (HP1) homolog, HPL-2, which contribute to the regulation of specific Hox gene expression through their binding to the repressive mark, H3K27me3. Furthermore, HIS-24 and HPL-2 act in a parallel pathway as members of the evolutionally conserved Polycomb group (PcG) silencing complex, MES-2/3/6. By microarray analysis, we found that HIS-24 and HPL-2 are not global transcriptional repressors as suggested by early studies, but rather are fine tuners of selected genes. Here, we discuss how HIS-24 and HPL-2 are responsible for the repression of specific genes in *C. elegans*. We suggest possible mechanisms for such an unanticipated function of an individual H1 variant and HP1 in the transcriptional repression of Hox genes.

C. elegans as a Model to Study the Establishment of Hox Gene Expression Patterns

The *C. elegans* Hox cluster consists of six genes that are expressed in restricted regions of diverse tissues and therefore

define region-specific differentiation characteristics.¹ The *ceb-13* gene is essential for anterior patterning,² *nob-1* and *php-3* are required for posterior patterning,³ and *lin-39* is necessary for development of vulva.⁴ The remaining two Hox genes, *mab-5* and *egl-5*, regulate development of posterior structures in males.⁵

To identify the components involved in the regulation of expression patterns of Hox genes, strains carrying the Hox reporter genes are used to screen for mutants with expanded ectopic Hox gene expression. In addition, genetic screens for identifying regulators of male tail neurogenesis, vulva or male tail development are performed.^{6,7} The expression pattern of Hox genes appears to be regulated by several conserved regulation pathways. For example, the expression of *egl-5* in the ventral neuroectoblast P12 is activated by the Wnt signaling and EGFP pathways,⁸ while in the embryonic muscle lineages, it is activated by VAB-7.⁹ Similar to mammals and *Drosophila*, the Hox genes of *C. elegans* are also globally repressed by Polycomb group (PcG) complexes, the Extra Sex Combs/Enhancer of Zeste (ESC/E(Z)) complex and Polycomb repressive complex 1 (PRC1).^{6,10,11} Mutations in *mes-2* and *mes-6* (maternal-effect sterile), which are the orthologs of the PcG genes *E(z)* and *esc*, respectively, cause ectopic expression of Hox genes as well as the presence of ectopic rays, ray fusions and changes in ray position.⁶ The ectopic expression of Hox genes has also been observed in the absence of *sop-2* or *sor-1* genes. SOP-2/SOR-1 form another PcG-like complex, which shares many structural and functional properties with the PRC1 complex, but they do not

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Table 1. Phenotype of individual linker histone variants or in combination with heterochromatin protein 1

Linker histone variant	Knock-down (KD) or knockout (KO)	Phenotype
HIS-24	KO KO and KD	increased H3K4 and decreased H3K9 tri-methyl mark at transgenes in the germline; ²⁹ defect innate immune system; ²⁸ positive and negative effects on specific gene expression; ^{17,28} embryonic lethality, reduced fertility, defects in germline development and differentiation, chromatin silencing ^{21,29}
HIL-1	KD	uncoordinated and egg laying defective animals ²²
HIL-2	KD	no obvious phenotype
HIL-3	KD	no obvious phenotype
HIL-4	KD	no obvious phenotype
HIL-5	KD	no obvious phenotype
HIL-6	KD	no obvious phenotype
HIL-7	KD	no obvious phenotype
HIS-24/HPL-1	KO	defect innate immune system; positive and negative effects on specific gene expression; ²⁸
HIS-24/HPL-2	KO	positive and negative effects on specific gene expression; defects in vulva cell specification, reduced brood size and fertility, defects in male tail development, ectopic expression of Hox genes (<i>mab-5</i> and <i>egl-5</i>) ^{17,28}
HIL-3/HPL-2	KO	no obvious phenotype in comparison with animals with <i>hpl-2</i> mutation ¹⁷

have orthologs in higher species, even in the very closely related *C. briggsae*.^{10,11}

A major advancement in understanding the mechanism of PcG-mediated gene silencing has been the discovery that E(Z) possesses methyltransferase activity for lysine 27 of histone H3.^{12,13} By depositing the H3K27me3 mark, the PcG complex maintains repression of Hox genes. Mutations in *C. elegans* PcG-related genes *mes-2* and *mes-6* cause the ectopic expression of Hox genes in regions where they should be silenced.^{6,15} The effects of the MES proteins in somatic patterning are subtle and the mechanisms by which the MES-2/3/6 complex is recruited to the Hox genes remains unknown.⁶ In contrast, the Trithorax group (TrxG) proteins maintain active expression by depositing H3K4me3 marks.¹⁴ In *C. elegans*, *trxG* mutants (*lin-49* and *lin-59*) fail to maintain expression in regions where they should normally be expressed.¹⁶ The bromodomain protein LIN-49 and trithorax-related protein LIN-59 are important in somatic development similar to the *Drosophila* *trxG* genes.¹⁶

Linker Histone Variant HIS-24 and Heterochromatin Protein 1 (HP1) as Gene-Specific Regulators

Recently, we have shown that the expression pattern of Hox genes can be regulated by chromatin factors, such as linker

histone H1 variant (*C. elegans* HIS-24) and the *C. elegans* HP1-like protein.¹⁷ H1 and HP1 are major chromatin structural proteins that have an important role in establishing and maintaining higher order chromatin structure. Both proteins form a complex family of related proteins with distinct species, tissue and developmental specificity.^{18,19} In vertebrates, these proteins physically interact through recognition of the methylated form of histone H1 by HP1.²⁰ Their cross-talk in regulating gene expression and epigenetic events during animal development is not well understood; however HP1 and H1 play important roles in heterochromatin packaging and gene regulation.

C. elegans possesses eight linker histone variants (HIS-24 and HIL-1 to HIL-7) and two HP1 homologs, HPL-1 and HPL-2. Depletion of individual linker histones, such as *bis-24* or *hil-1* and *hpl*, results in specific phenotypes (see Table 1). The *bis-24* loss-of-function mutant has severe defects in germline proliferation and differentiation.²¹ Lack of *hil-1*, which is an untypical linker histone variant, results in uncoordinated and defective egg laying in animals.²² Single *hil-2* to *hil-7* knockout animals are viable with no obvious phenotype (unpublished data). The lack of a phenotype in these mutants can be due to compensation by the remaining HIL subtypes, which can be similar to the situation observed in mice and other organisms.^{23,24}

Deletion of the *C. elegans hpl-2* gene results in temperature-sensitive non-lethal developmental abnormalities, such as the multivulva phenotype and desilencing of repetitive arrays in the germline.^{25,26} The second *C. elegans* HP1 homolog, *hpl-1*, acts redundantly with *hpl-2* in postembryonic development.²⁷ Similar to the situation in vertebrates, HPL-1, but not HPL-2, recognizes the methylated form of HIS-24.²⁸ Therefore, due to an evolutionary conserved interaction between H1 and HP1 as well as the lack of vertebrate knockout alleles or proper tools, we decided to study the biological role of HP1 and H1 crosstalk in chromatin-mediated processes in *C. elegans*. By microarray analysis, we found that elimination of HIS-24 and HPL-2 results in the regulation of a small number of genes (ca. 7%), although histone H1 and HP1 play a role in chromatin structure, organization and compaction.¹⁷⁻¹⁹ We also found that the lack of HIS-24 (but not HIL-3) and HPL proteins did not change global levels of repressive core histone H3 methylation marks at lysine 9 and 27, but did affect chromatin compaction in the germline. Since alterations of heterochromatin-associated histone marks such as H3K27me3, H3K9me2 or H4K16ac (acetylation at lysine 16) are associated with the presence of H1 in many organisms,²⁹⁻³¹ we cannot exclude the possibility that HIS-24 and HPL proteins influence the level of

histone modifications at specific loci, in distinct type cells or at certain developmental stages of *C. elegans*. Our further studies showed that depletion of HIS-24 caused increased levels of the activating histone H3 lysine 4 methylation mark with a concomitant decrease of the repressive histone H3 lysine 9 methylation mark at the transgenes in the germline.²⁹ In vertebrates, a triple H1 knockout in embryonic stem cells leads to a 2-fold reduction in H3K27 methylation.³⁰ In *Drosophila*, H1 is required for heterochromatin structural integrity as well as the deposition or maintenance of major pericentric heterochromatin-associated histone marks, including H3K9Me2 and H4K20Me2.³¹

Furthermore, our microarray data analyses of *his-24*; *hpl-2* double and *his-24* *hpl-1*; *hpl-2* triple mutant animals revealed differential expression of genes involved in the embryonic or male tail development and reproduction and were consistent with the phenotypic defects of the mutant animals. Animals lacking HIS-24 and HPL-1/2 are viable with synergistic effects on vulval cell fate specifications, sterility, reduced brood size or positional identity in males. This modest change in expression of just a small number of genes in triple mutant animals *his-24* *hpl-1*; *hpl-2* was unexpected, but can be explained by a specific role of the individual H1 variant, HIS-24 and HPL proteins in the control of particular gene expression. Elimination of H1 in *Tetrahymena*³² or in yeast³³ also resulted in the regulation of specific genes.

Linker Histone HIS-24 with HPL-2 Influence the Expression of Hox Genes and Male Tail Development

We previously reported that the *his-24*; *hpl-2* double mutant animals show abnormalities in patterning and development of rays, while *his-24* *hpl-1* or *hpl-2*; *hpl-1* double mutants have normal development of rays.¹⁷ Our observations indicated that wild-type *hpl-2* and *his-24* are required for repressing the expression of two Hox genes (*egl-5* and *mab-5*) outside of their normal expression domains through HIS-24 binding to the promoters. However, to date we have failed to detect HPL-2 at the regions of *egl-5* and *mab-5* Hox

genes using a ChIP approach, and therefore we speculate that HPL-2 can regulate the expression of these genes indirectly through RNAi machinery, transcription factors or distinct posttranslational modification profiles. We found that HIS-24 and HPL-2 bind the repressive chromatin mark H3K27me3 and that loss of HPL-2 and HIS-24 together with depletion of MES-2 (a member of the PcG proteins) resulted in additive defects in male tail development. Although we currently cannot explain why HIS-24 and HPL-2 have this unusual binding behavior, we hypothesize that in the context of binding to Hox genes, the H3K27me3 mark is important. In addition, the association of HPL-2 with H3K27me3 suggests that the protein has sufficient plasticity to recognize and bind tri-methyl marks of H3K9 and H3K27. HP1 is known to specifically bind to H3K9me3.³⁴ Furthermore, the chromodomain of HP1 has similarities to that found in several protein members of the PRC1 complex, such as CBX2 and CBX7, which are known to directly interact with H3K27me3 and H3K9me3.³⁵ Therefore, we propose that HIS-24 and HPL-2 are part of the PcG silencing complex at specific Hox genes and that both proteins functionally interact. To determine how the HIS-24 variant discriminates between histone modification marks, we assessed its distinct posttranslational modification profile. Interestingly, the lysine methylation form of HIS-24 was shown to bind the H3K27me3 chromatin mark and rescue male tail development in *his-24*; *hpl-2* double mutant animals. Therefore, these results allow us to propose a model whereby the methylated form of HIS-24 is enriched at the promoters of transcriptionally repressed genes as well as regulatory regions, and together with HPL-2, can allow local silencing, thus facilitating access for transcriptional repressors (Fig. 1). Another possibility is that the methylated form of HIS-24 together with HPL-2 could have a stronger affinity for chromatin, thereby creating a more closed/silenced chromatin environment at distinct loci.

Potential Molecular Mechanisms

Our study has provided the first example of H1 and HP1 influence on Hox gene

expression through their binding to the repressive form of H3, H3K27me3. In mammals, H1 has been shown to regulate Hox gene expression by promoting DNA methylation³⁶ and activating, rather than repressing, the expression of Hox genes in mouse embryos,³⁷ although chromatin decompaction and changes in 3D chromatin architecture coincide with the activation of Hox genes during embryonic development.³⁸⁻⁴⁰ In mice, deletion of individual H1 subtypes results in the downregulation of specific Hox genes in embryonic stem cells (ESC), which correlates with a reduction in both the expression and level of an active H3K4me3 mark.³⁷ In contrast, occupancy of the repressive H3K27me3 mark was modestly increased at specific Hox genes, but the global level of this histone modification was reduced.⁴¹ Therefore, the opposing role of H1 in regulating Hox gene expression in *C. elegans* and mice could be due to different posttranslational modification and/or transcription factors binding to H1 and therefore modulating the effects of H1 on gene silencing. In vertebrates, the dual (active or repressive) role of H1 has been shown to depend on its posttranslational modifications as well as on interacting factors.⁴² Additionally, the upregulation of Hox genes in *his-24*; *hpl-2* depleted *C. elegans* reflects the expression of Hox genes in organisms at later stages of life and not in embryos. Interestingly, the opposing role of factors regulating Hox gene expression in *C. elegans* and mice has also been observed for the transcription factor NFY, which specifically recognizes the CCAAT box of the *egl-5* gene.⁴³

H1 has been shown to stimulate the activity of PRC2 in vitro toward the methylation of H3K27me3 when H1 is incorporated into nucleosomes.^{44,45} However it was unclear whether HIS-24 and/or HPL-2 help to maintain the repressive H3K27me3 mark or whether they are simply recruited to the promoter through recognition of H3K27me3 (Fig. 1). We postulate that loss of HIS-24 and HPL-2 may affect binding of polycomb complex components to the Hox genes and therefore can positively influence the recruitment of Trithorax (TrhG) group. This can lead to the ability of histone methyltransferases to trimethylate H3K4

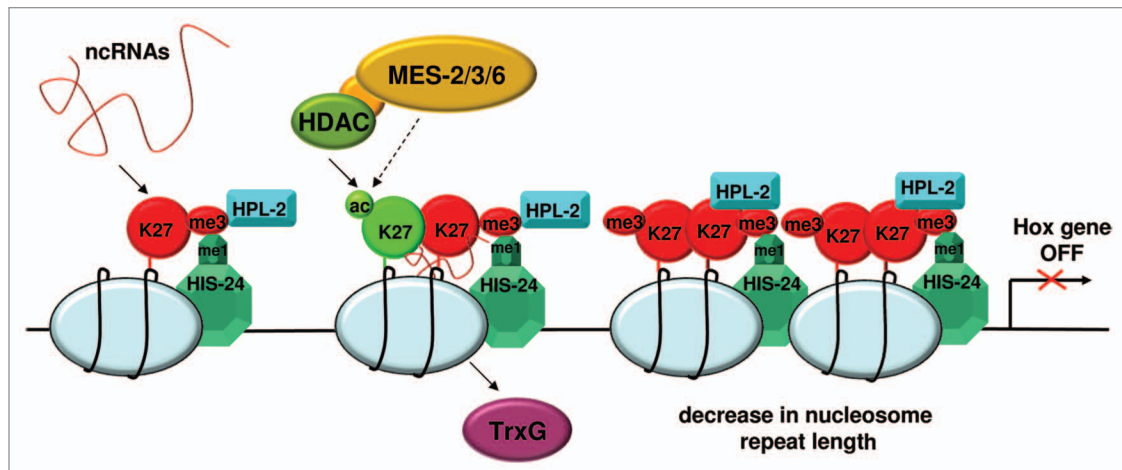


Figure 1. Model explaining how the methylated form of HIS-24 (HIS-24me1) and HPL-2 can have stimulatory effects on Hox gene repression. HIS-24me1 and HPL-2 binding to H3K27me3 can recruit coding and non-coding RNA (ncRNAs) molecules and positively influence the binding of the repressors such as histone deacetylase proteins HDACs (HDAC-1, HDAC-2) to distinct genomic regions. *C. elegans* PcG-related complex (MES-2/3/6) can transiently interact with HDACs to adjust the local histone code for silencing.¹³ HDACs could deacetylate H3K27 to make the ϵ -amino group available for methylation by PcG. The methylation of H3K27me3 results in release of TrxG complex and the reduction of the H3K4me3 signal. Additionally, the interaction of methylated forms of HIS-24 and HPL-2 with H3K27me3 can cause changes in nucleosome positioning or repeat length.

(active chromatin mark) and/or can result in alterations in the H3K27 trimethyl marks. Whether the activation of Hox gene expression in a *his-24*; *hpl-2* mutant background is correlated with dynamic patterns of H3K27me3 and/or H3K4me3 marks in the distinct cell type remains to be elucidated. Another possibility for HIS-24 and HPL-2 in regulating Hox gene expression could be through changes in nucleosome positioning or in the nucleosome repeat length at specific loci, which is similar to the situation in vertebrates lacking H1.³⁰ In *C. elegans*, we observed lower levels of histone H3 occupancy at the *mab-5* promoter in a *his-24* mutation background compared with wild-type animals, suggesting that the differences of H3 levels could be due to the nucleosome free region that forms at high levels of expression.¹⁷ Furthermore, the distance between enhancers or regulatory regions of Hox clusters could be altered by a loss of HPL-2 and HIS-24. Finally, at this stage, we cannot exclude the possibility that the methylated forms of HIS-24 and HPL-2 may recruit other proteins as well as coding or non-coding RNA molecules to potentiate the binding of other repressors, such as histone deacetylase proteins, which would promote the establishment and/or maintenance of repressive epigenetic modifications and silence the expression of Hox genes (Fig. 1). Another

question of interest is how HIS-24- and HPL-2-mediated repression is relieved in cells that normally express Hox genes, since *his-24* and *hpl-2* appear to be widely expressed in all cells.^{17,25,27}

Concluding Remarks

Our results in a recently published study establish a novel link between the methylated form of the linker histone and HP1 in Hox gene regulation. Our recent knock-out studies and biochemical analyses have revealed exciting new insights into specificity and mechanisms of action of the individual H1 and HP1 proteins. These proteins are not mere structural components of chromatin and general repressors of transcription, but also play a role in controlling specific gene expression in a negative and positive way, and therefore contribute to the specific regulation of chromatin-related processes. H1 and HP1 are redundant in their ability to compact chromatin globally and regulate a small subset of specific genes at the level of local chromatin organization. The methylated form of HIS-24 could be considered as an epigenetic mark, which is specifically enriched at distinct genomic regions for establishing the repressive pattern of selected homeotic genes in different cell types and at different developmental stages.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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