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Interplay between histone deacetylase SIR-2, linker histone H1 and histone methyltransferases in heterochromatin formation

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Maintenance of intact heterochromatin structure through epigenetic mechanisms is essential for cell survival. Defects in heterochromatin formation caused by loss of chromatin-modifying enzymes lead to genomic instability and cellular senescence. The NAD$^+$-dependent histone deacetylase SIR-2 and the H1 linker histone are intriguing chromatin elements that are connected to chromatin regulation and cell viability in the single cellular eukaryotic organism yeast. However, it remains an open question how SIR-2 and H1 mediate heterochromatin formation in simple multi-cellular organisms such as *C. elegans* and in even more complex organisms such as mammals. Recently we have identified SIR-2.1 and the H1 histone subtype, HIS-24 as factors involved in heterochromatin regulation at subtelomeric regions in *C. elegans*. In addition we show that SIR-2.1, HIS-24 and MES-2, a ortholog to Enhancer of zeste E(Z) are functionally related in heterochromatin formation contributing to fertility and embryogenesis. Here we discuss the interplay between SIR-2, H1 histone and histone methyltransferases in modulation of chromatin structure in further detail.

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Introduction

Compaction of chromatin in higher-order structures plays a central role in the regulation of gene expression and genome stability.[1,2] At telomeres and subtelomeres, transcriptionally silent heterochromatin stabilizes chromosomal ends and ensures proper mitotic segregation.

Formation of heterochromatin is mediated by particular core histone modifications (hypoacetylation and methylation) and binding of H1 linker histones and non-histone chromatin factors.[1-3]

Key regulators in heterochromatin formation are silent information regulator 2 proteins—SIR-2 (also referred to as sirtuins) which were first identified in *S. cerevisiae* to be essential for gene silencing not only at telomeres but also the silent mating type loci (HM loci) and ribosomal DNA (rDNA).[3,4] SIR-2 proteins are highly conserved NAD$^+$-dependent histone deacetylases that catalyze a unique reaction which couples deacetylation of lysine residues to hydrolysis of NAD$^+$.[4,5]

In recent years there has been growing evidence that deacetylation of histones by sirtuins is closely associated with histone methylation in heterochromatin formation and this functional relationship seems to be conserved from yeast (*S. pombe*) to human.[6-10] In human, it was shown that histone methylation is also regulated through linker histone H1.[11-14]

The H1 linker histone and its variant forms have been implicated in the formation of higher-order chromatin structures and gene repression. Binding of H1 to nucleosomes stablizes the nucleosomal particle structure and helps folding the chromatin fiber into higher-order structures.[15]

In our recent study we observed that *C. elegans* SIR-2.1 together with linker histone HIS-24 and histone methyltransferase (HMT) MES-2 (Maternal effect sterility-2) is involved in maintenance of H3K27 methyl mark at subtelomeric regions.[16,17] MES-2 is an ortholog to the Drosophila Polycomb group (PcG) protein

Enhancer of zeste [E(Z)] and a member of a Polycomb-like chromatin repressive complex (PRC).[17]

HIS-24 specifically interacts with H3K27me3 in the germ line of *C. elegans* and promotes together with SIR-2.1 the modification of H3K27me3 at subtelomeric regions. Until now an interplay between histone deacetylase SIR-2, E(Z) and linker histone H1 in the formation of heterochromatin has not been shown.

Here we will discuss the link between the histone deacetylase SIR-2 and linker histone H1 to H3K27 histone methylation and heterochromatin formation. We will bring our observations in context with current knowledge on this evolutionarily conserved relationship.

Cross-Talk Between Histone Deacetylases and Methyltransferases During Heterochromatin Formation

Interplay between the histone H3K9 methylation—an evolutionarily stable heterochromatin mark—and histone H3K9 deacetylation by SIR-2 proteins has been demonstrated in several eukaryotic organisms.[7-9] In *S. pombe* SIR-2 specifically deacetylates H3K9ac, which is a prerequisite for methylation of this H3 residue by Crl4, a SET domain-containing histone methyltransferase.[18] Consequently Heterochromatin protein 1 (HP1) (Swi6 in *S. pombe*) binds to H3K9 and helps to establish heterochromatin.[19,20]

In mammals, depletion of SIR-2 homologue SIRT1 by RNAi results not only in a global increase in H4K16ac and H3K9ac but also in a loss in H3K9me3 and H4K20me1.[6,7,10] SIRT1 was shown to directly interact with Supressor of variegation 3–9 homoloque 1 (SUV39H1) and to promote H3K9 methylation through deacetylation of H3K9ac and SUV39H1K266 in its SET-domain.[7]

Deacetylation of H4K16ac and H3K9ac by SIRT1 also results in a direct recruitment of H1.4 histone, a key factor in heterochromatin formation.[11] SIRT1 deacetylates H1.4 at lysine 26 and H1.4K26 can then be methylated by the Polycomb protein EZH2, a homolog of Drosophila E(Z).[11-13,21] Interestingly, SIRT1 and EZH2 coexist in the PRC4 complex. Thus, deacetylation of H1K26ac could be coordinated with methylation by EZH2.[13]

In contrast to yeast and mammals, in *C. elegans* H3K9 deacetylation by SIR-2 has not been reported to be related to H3K9 methylation. In *C. elegans*, H3K9 methylation was cytologically detected on the X chromosome and telomeres, but heterochromatin has not been defined.[22] Additionally, *C. elegans* SU(VAR)3-9-like proteins exhibiting incompletely conserved chromo and SET domains are not able to methylate H3K9.[23]

In our study, we observed that deacetylation of H3K9ac by SIR-2.1 at subtelomeric regions induces together with linker histone HIS-24 H3K27 methylation in the *C. elegans* germ line. Indeed H3K9ac and H3K27me3 exclude each other at the subtelomeric regions. This implicates an intimate link between histone deacetylase SIR-2.1 and H3K27 HMT complex (MES) that also involves linker histone H1.[16] Interestingly, we did not observe any changes in H3K9me2 levels in *sir-2.1* mutant worms suggesting that deacetylation of H3K9ac is only a prerequisite for H3K27 methylation in the *C. elegans* germline.

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In *C. elegans*, one of the three MES complex proteins, MES-2, a homolog of E(Z) possesses HMT activity and mediates di- and trimethylation of H3K27.[17] MES-2 containing a conserved SET-domain, has robust HMTase activity, which depends upon both MES-6, an Extra sex combs (ESC) homolog, and MES-3, a pioneer protein unrelated to PcG proteins of other organisms.[24] The MES complex regulates gene silencing, germ line development and is responsible for H3 K27 methylation in most regions of the germline and in early embryos.[17] This resembles functionally Polycomb-group genes in Drosophila.[16,25,26] Nevertheless there are major differences in the subunit composition of mammalian PRC and fly PcG complexes. These complexes consist of SET domain-containing EZH2/E(Z) and three non-catalytic subunits SU(Z)12, EED/ESC and RbAp46/48/p55. The worm MES complex lacks a SU(Z)12 homolog and the histone binding protein p55 (**Fig. 1**). This implicates distinct strategies for the formation of active HMTase complexes between worms, flies and mammals.[24]

Based on our study, we suppose that SIR-2.1 and HIS-24 are part of the PcG silencing complex in the *C. elegans* germ line and that these factors functionally and enzymatically interact. Drosophila SIR-2 (DmSIR-2) interacts with PcG genes, as mutations in DmSIR-2 synergistically enhance the phenotype of PcG mutants.[27] Both DmSIR-2 and SIRT1 physically associate with E(Z)[27] and EZH2,[13] respectively, and might regulate their enzymatic activity. Interestingly the lysine residue K266 of SUV39H1 is also conserved in the SET-domains of E(Z) and EZH2 suggesting direct deacetylation by SIR-2, rendering them more active and promoting H3K27me3 and H1.4K26me2/3 (**Fig. 2**).[7]

Therefore enzymatic modification of HIS-24 by SIR-2.1 and MES-2 as well as deacetylation of MES-2 by SIR-2.1 (**Fig. 2**) are intriguing possibilities to explain their functional relationship in respect to fertility and brood size in *C. elegans*.

In germ cells the majority of HIS-24 is located in the cytoplasm. Since this unusual localization depends on MES complex proteins and SIR-2.1, we speculate that this "non-nuclear localisation" of HIS-24 might be linked to its post-translational modifications by SIR-2.1 and MES.[28]

Lack of HIS-24 and SIR-2.1 causes a loss of H3K27me3 in *C. elegans* germ line similar to lack of HIS-24 and MES-3 as previously reported.[16,28] Double mutant *sir-2.1;his-24* or *mes-3;his-24* worms displayed sterility, decreased brood size and increased numbers of dead embryos,[16,28] suggesting that the observed phenotypes correlate with loss of H3K27me3 in germ line nuclei.

In mammalian embryonic stem cells a triple H1 knockout leads to a two-fold reduction in H3K27 methylation, and deficiency in three somatic H1 variants causes embryonic lethality in mice.[29,30] All these facts indicate a conserved mechanism present in both invertebrates and vertebrates.

We hypothesize that SIR-2.1, HIS-24 and MES function as a complex in the same developmental pathways regulating heterochromatin formation through

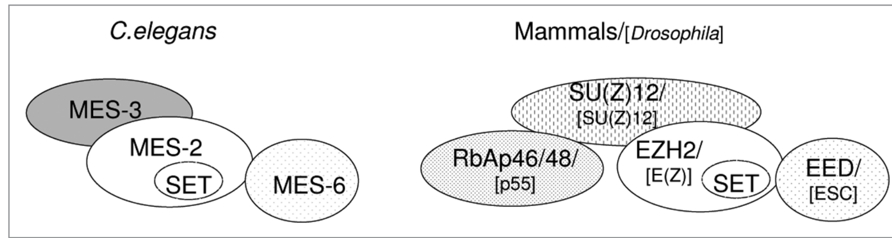

Figure 1. The Polycomb Group (PcG) complexes are evolutionarily conserved and required for normal development and fertility. The mammalian Polycomb repressive complex (PRC) contains four core subunits: EZH2 (homolog of Drosophila Enhancer of zeste [E(Z)]), EED (Embryonic ectoderm development, a homolog of ESC (Extra sex combs)), and SU(Z)12 (Suppressor of zeste-12) stabilized by RbAp46/48 (p55 in Drosophila). The *C. elegans* PcG complex is built from three subunits: MES-2 (Maternal effect sterility-2), MES-3 and MES-6. MES-2 is a homolog of E(Z) with HMTase activity, which depends on the two non-catalytic subunits: MES-6, an ESC homolog, and MES-3, a novel protein unrelated to PcG proteins from other species. Interestingly, a SU(Z)12 homolog has not been identified in *C. elegans*. Homologs of Drosophila PcG proteins are given in brackets.

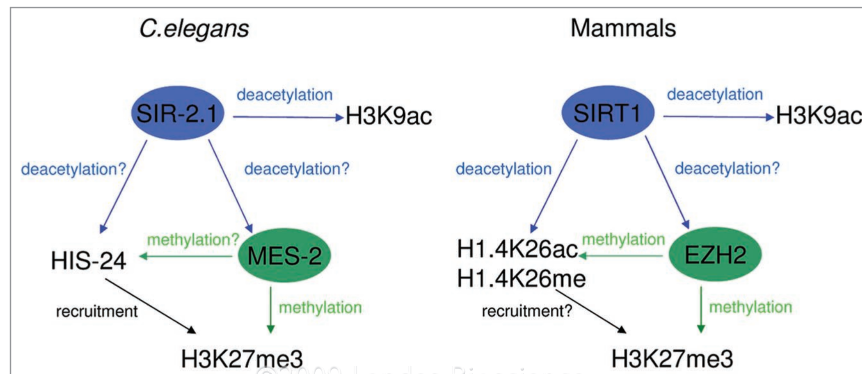

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Figure 2. Suggested model for the conserved interplay between histone deacetylase SIR-2, linker histone H1 and histone methyltransferase MES-2/EZH2 in *C. elegans* and mammals. Deacetylation of linker histone HIS-24/H1.4, H3K9ac and a conserved lysine residue in the SET domain of MES-2/EZH2 (green) by SIR-2.1/SIRT1 promotes methylation of H3K27 and HIS-24/H1.4 recruitment to heterochromatin.

H3K9 deacetylation and H3K27 methylation in the *C. elegans* germ line. This idea is reinforced by the following facts: first, HIS-24, SIR-2.1 and MES proteins are involved in germ line silencing.[31] Second, H1 promotes H3 methylation in *C. elegans* as well as represses core histone acetylation in mammals.[28,31,32] Third, the majority of HIS-24 and H3K27me3 colocalization is found close to the nuclear membrane in electron-dense regions of germ line cells that cytologically define heterochromatin.[16] Further studies are required to understand the molecular and enzymatic relations between SIR-2.1, PcG proteins and linker histone HIS-24 in *C. elegans*.

Perspectives/Future Directions

It has been shown that induced silencing by SIR-2 participates in position effect variegation (PEV) in Drosophila.[34] Currently, there is no genetic evidence supporting PEV in *C. elegans*. Nevertheless, in our study we could show that SIR-2.1 associates with and enzymatically modifies telomeric chromatin and thereby positively regulates telomeric methylation by recruiting linker histone HIS-24.[16] Interestingly, HIS-24 and SIR-2.1 introduced in *S. cerevisiae* can affect telomere position effect variegation (TPEV) suggesting that similar epigenetic silencing mechanisms might exist in worms and flies, and are controlled by histone H1 and SIR-2.[31]

Another intriguing question arising from our work is whether chromatin regulation mediated by SIR-2, linker histone H1 and PcG proteins affects life span and aging-related processes in *C. elegans* and other organisms. It has been shown that the germ line of the nematode *C. elegans* influences life-span.[33] Therefore it is tempting to speculate that defects in establishment or maintenance of chromatin modifications such as H3K27me3 in the *C. elegans* germ line alter gene expression and silencing and thereby ultimately affect life span.

Future comprehensive analyses combining biochemistry and genetics with genomic and proteomics approaches in *C. elegans* and other organisms will provide further insights to these remaining questions.

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