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REVIEW

Analysis of the Hox epigenetic code

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Abstract

Archetypes of histone modifications associated with diverse chromosomal states that regulate access to DNA are leading the hypothesis of the histone code (or epigenetic code). However, it is still not evident how these post-translational modifications of histone tails lead to changes in chromatin structure. Histone modifications are able to activate and/or inactivate several genes and can be transmitted to next generation cells due to an epigenetic memory. The challenging issue is to identify or "decrypt" the code used to transmit these modifications to descent cells. Here, an attempt is made to describe how histone modifications operate as part of histone code that stipulates patterns of gene expression. This papers emphasizes particularly on the correlation between histone modifications and patterns of *Hox* gene expression in *Caenorhabditis elegans*. This work serves as an example to illustrate the power of the epigenetic machinery and its use in drug design and discovery.

Key words: Epigenetic code; Histone code; Histone modifications; *Hox* gene expression

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INTRODUCTION

The basic unit of chromatin corresponds to DNA that is packaged into periodic nucleoprotein structures known as nucleosomes[1]. The nucleosome comprises an octamer of eight core histone proteins (two H2A, H2B, H3 and H4) around which 146 base pairs of dsDNA are wrapped in 1.65 left-handed superhelical turns[2]. Histone H1 serves as a linker protein and directs the formation of a higher-order structure in the nucleosomal array. The histone N-terminal tails comprise between 25 and 40 residues and are exposed on the surface of the nucleosome. The amino acid sequences of these N-terminal tails are highly conserved, possibly due to the roles played by a number of important post-translational modifications at these sequences[3].

A number of selected amino acid residues are subject to a variety of enzyme-catalyzed posttranslational modifications. These modifications include acetylation and methylation of lysines (K) and arginines (R), phosphorylation of serines (S) and threonines (T)[4,5], which are carried out by a variety of chromatin modifying complexes, such as the COMPASS (for histone methylation), NuA4/Tip60 (for Histone H4 acetylation), and NuA3 (for Histone H3 acetylation) complexes. All of these chromatin modifying complexes contain one of the histone modification enzymes, such as histone acetyltransferase (HAT), histone deacetylase (HDAC), histone methyltransferase (HMT), histone demethylase (HDMT), and histone kinase. These chromatin modification complexes work in concert with ATP-dependent chromatin-remodeling complexes, including the SWI/SNF, ISWI and NURD/

Mi-2/CHD complexes, which recognize specific histone modifications to restructure and mobilize nucleosomes.

Histone tails represent a complex set of epigenetic information. There are 50 distinct acetylated isoforms of the eight histone proteins[6]. In addition, several modifications can be applied to these isoforms including methylation of selected lysines and arginines (H3 and H4) and phosphorylation of serine (H3, H4, H2B). The methylation process includes the attachment of one, two, or three methyl groups. Other histone tail modifications also include ubiquitination and ADP-ribosylation[7]. The nucleosome surface is then decorated with thousands of these modifications, which could comprise a histone code[8,9] or an epigenetic code[10].

A challenging development occurs when a cell proliferates and generates two identical cells containing genes having the same status (expressed or repressed) as the ones in the mother cell. Every cell of an organism follows the same genetic code except germ cells and some cells of the immune system. Hence, the regulation of gene expression is not exclusively controlled by DNA but it is conducted in harmony with histones[11].

Cells control processes that permit them to remember the status of each gene before mitosis, and therefore preserve its phenotype. This transmission of gene expression patterns from mother cells to its descendants occur through a mechanism of gene bookmarking[12]. Hence, the cell state (identity) is kept safe within the structure of the chromatin and the epigenetic code.

Components of chromatin including DNA and histones undergo dynamic post-synthetic covalent modifications. The dynamic and not permanent post-translational modifications on histones represent epigenetic signatures and are created and removed whenever needed to alter the expression states of loci. These marks involve activities of modifying enzymes (writers), enzymes removing modifications (erasers), and readers of the epigenetic code. The erasers are crucial targets for manipulation to further understand the histone code and its role in biology and human disease[13,14].

The inheritance of epigenetic information is orchestrated by histone code readers (proteins that identify particular histone modifications) and histone code writers (proteins that duplicate the histone modifications).

Histone code readers and writers include structural domains such as the bromodomain, the chromodomain, and the plant homeodomain (PHD)[15]. These domains are required to recognize specific patterns of histone modifications including acetylation and methylation at given locations. The bromodomain is located mainly in HATs and chromatin remodeling proteins, whereas the chromodomain is found for example in HATs, HMTs, and HP1 (Figure 1). It has been shown that proteins involved in writing epigenetic information collaborate to maintain an epigenetic stability in the midst of great dynamic events at the molecular level[16].

Despite their phenotypic differences, *Caenorhabditis elegans* (*C. elegans*), a roundworm with a genome about 30

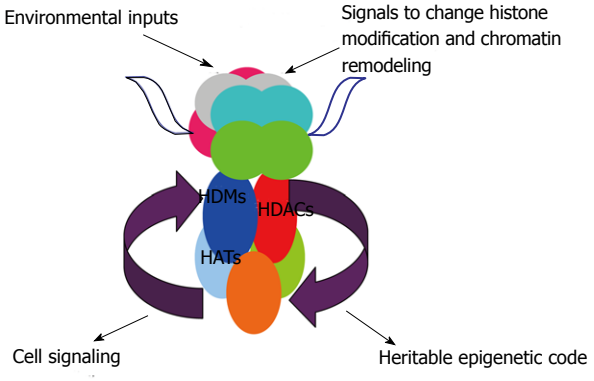

Figure 1 Histone modifications and chromatin remodeling. Environment factors and modifying enzymes are associated with regulating cell signaling and histone code. HDAC: Histone deacetylase; HAT: Histone acetyltransferase.

times smaller than human genome, however it encodes 22 000 proteins. In addition, approximately 35% of *C. elegans* genes are closely related to human genes, and both organisms have at least 80% amino acid sequence identity between their core histones[17]. For example, MES-2, the ortholog of human EZH2 has been reported to be a HMT for H3K27[18], and MES-4, a SET domain containing protein, has recently been shown to be required for H3K36 di-methylation (H3K36 me2) in mitotic and early meiotic germline nuclei and in early embryonic cells[19]. Whetstine *et al*[20] discovered the histone demethylase JMJD2A in mammalian cells and that has led to the identification of the *C. elegans* homolog, JMJD-2. This protein family was reported to be required in chemical methylation for H3K9/k36 me3.

There is accurate machinery that allows cells to recognize themselves and undertake specific tasks. This machinery represents the blueprint of various patterns of gene activation/inactivation throughout the cell cycle. Lack of expression or repression leads to an irregular outcome for the cell including altered genetic programs and increased rate of cell transformation[12]. This "know-how" is located mainly in the amino-terminal tails of the core histones[21]. The first association between a histone tail modification and a particular functional state of chromatin was reported by Pogo *et al*[22] and Hebbes *et al*[23]. It was shown that transcriptionally active chromatin fractions are enriched in acetylated histones, whereas regions of facultative heterochromatin and transcriptionally silent constitutive were located in underacetylated regions[24] .

As depicted in Figure 1, the set of histone tail modifications includes at least two subsets. The first subset represents the modifications that lead to on-going transcription and usually are classified as cell signaling, and the second subset represents the modifications that are heritable. This heritability of transcriptional states is the component that unambiguously identifies the histone code[19]. In addition, these histone modifications are also suggested to be used combinatorially to instruct genes for activation right after cellular differentiation[25,26]. This latter proposition could be used to model the pro-

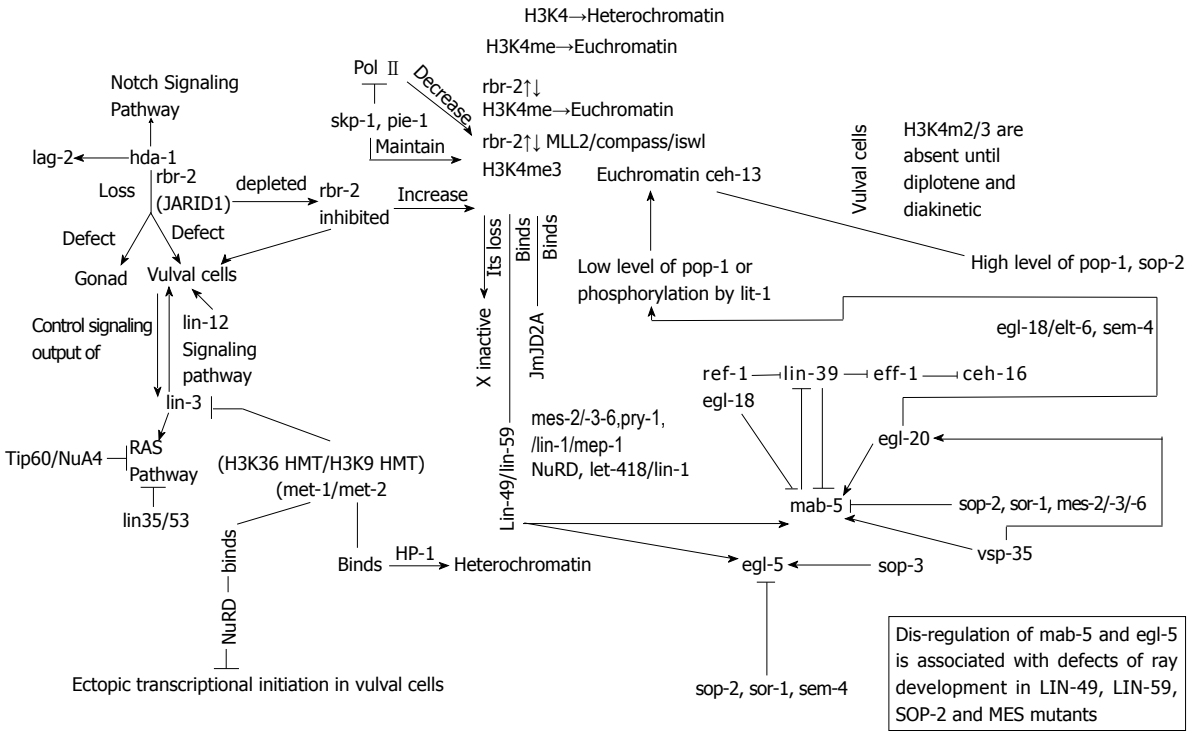

Figure 2 H3K4 modifications, Hox genes and cellular development.

Table 1 Methylation of H3K4, H3K9, H3K27 and H3K36

i	H3K4me(i)	H3K9me(i)	H3K27me(i)	H3K36me(i)
0	Off	On	On	Off
1	On	Off	Off	On
2	On	Off	Off	On
3	On	Off	Off	On

i=0 the me(i) part will be equal to zero which means that the histone is not methylated. Here the "On" state represents the transcriptionally euchromatin; and the "off" state represents the constitutive heterochromatin.

grammed activation of tissue-specific transcription factors throughout differentiation of ES cells[24].

Histone modifications associate closely with various biological functions. For example, as is depicted in Table 1, in *C. elegans*, methylation of H3K4/K36 correlates with transcriptionally competent euchromatin. Alternatively, methylation of H3K9/K27 correlates to a component of constitutive heterochromatin. Finding these patterns and the corresponding correlations with the transcriptional status of selected genes will lead the way to illustrate the process of an epigenetic code.

FROM *HOX* GENES TO HISTONE CODE

This study provides a detailed analysis of the Hox epigenetic code mainly in *C. elegans*. Methods used to propose such a code are based on a manual data mining approach and a thorough analysis of data gathered from various references and websites. A few discrepancies and contradictions were encountered during the design of Figures 2-4. For example, it is mentioned in a number of repositories for C. elegans that Sem-4 inhibits Lin-39. However, after investigating this issue further using the available literature and corresponding with many scientists, Sem-4 has been found to have an opposite role. Thus, patterns generated from data mining software should be manually checked to avoid similar discrepancies.

Although the histone code defined here targets a small organism, chromatin modifications in mammals including humans were used in this work to imply significant components of the Hox epigenetic code in *C. elegans*. In addition, there are many complexes that exist in both C. elegans and humans such as micro RNAs lin-4 and let-7 which have been connected to many cancers[27], and 153 kinase subfamilies which direct most cellular processes, particularly in signal transduction and coordination of complex pathways[28]. Similarities and homologs between both organisms are shown in Tables 2-5. Table 2 shows examples from the Ras-superfamily GTPases[29], Table 3 focuses on autophagy-related genes[30], Table 4 illustrates examples from the Ubiquitin-conjugating enzymes[31], and Table 5 shows specific similarities in *Hox* genes[32].

The development and maintenance of cellular identity is crucial in both embryonic and adult tissues for normal organ function. Hence the need to establish a stable transcriptional states within the cell, a process in which transcription factors have a vital role. One of those groups of transcription factors are known as *Hox* genes, representing a family of homeodomain-containing transcription factors that establish cellular identity during development, in addition to regulating numerous processes including apoptosis, receptor signalling, differentiation, motility and angiogenesis.

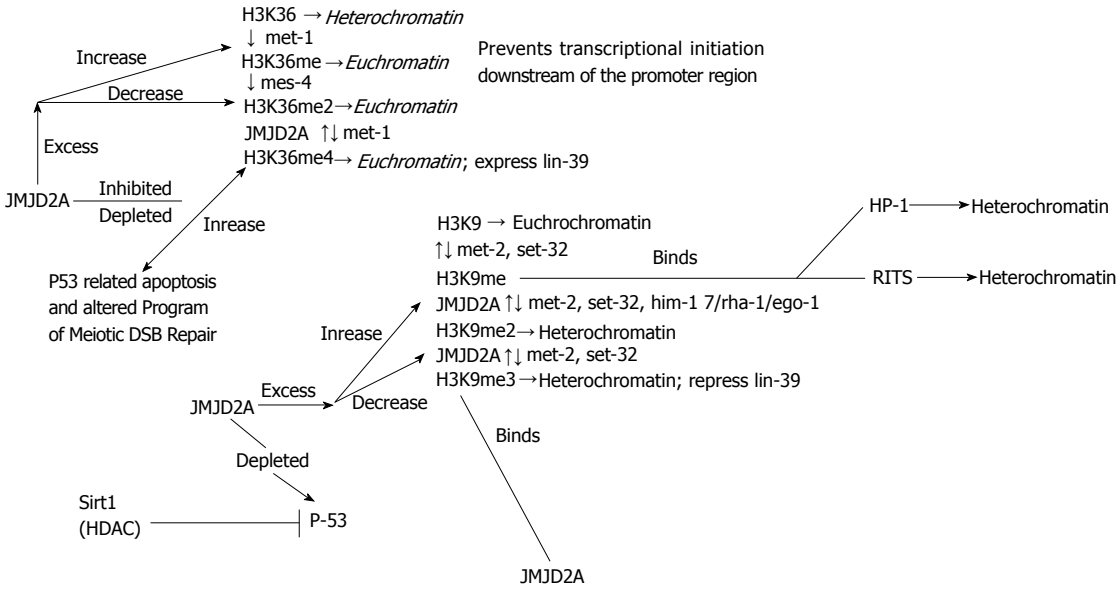

Figure 3 H3K36 modifications and cellular development. HDAC: Histone deacetylase.

Table 2 Known roles of Ras-superfamily GTPases in *Caenorhabditis elegans*: Homologs in humans

C.elegans gene name	Human homolog	Developmental role(s)
Ras/Rap/Ral family		
let-60	K-Ras	Cell fate determination, oocyte meiotic maturation, sex myoblast migration, axon pathfinding, olfaction, response to microbacterium nematophilum infection
Rho family		
rho-1	RhoA	Cytokinesis, hypodermal contraction, P cell migration, axon pathfinding
cdc-42	Cdc42	Cytokinesis, embryonic polarity, axon pathfinding
ced-10	Rac1	Gastrulation, cell migration (P cells, distal tip cells), cell corpse phagocytosis, neuronal migration, axon pathfinding, vulval morphogenesis
rac-2	Rac1	Neuronal migration, axon pathfinding
mig-2	Mtl	Cell migration (P-cells, distal tip cells, Q cells and descendants), neuronal migration, axon pathfinding, vulval morphogenesis
Rab family		
rab-1	Rab1	Innate immunity gene expression
rab-3	Rab3	Synaptic vesicle trafficking and release
Arf/Sar family		
arl-6	Arf6	Ciliary vesicle trafficking and Bardet-Beidl syndromes
evl-20	Arf2	Microtubule organization, vulval cell division, tissue morphogenesis
Ran family		
ran-1	Ran	Nuclear trafficking, nuclear reassembly, Kinetechore association with the mitotic spindle

Table 3 *Caenorhabditis elegans* orthologs of mammalian autophagy-related genes

C. elegans gene	Mammalian gene	Function
erp-1	*Bif-1*	Bax interacting factor that associates with Uvrag and Beclin 1
C33A11.4	*DRAM*	*p53*-induced damage-regulated
ced-9	*Bcl-2*	Anti-apoptotic protein, negative regulator of Beclin 1-mediated autophagy

Mammals have 39 *Hox* genes split between four groups of linked genes on different chromosomes and patterns of their deregulated expression have been reportered in cancer. This deregulation is tissue-specific, certain *Hox* genes that normally have tumour suppressive effects are silenced; and in other tissues, particular Hox genes are expressed in an abnormal temporospatial pattern with oncogenic effects[33]. Anomalies in *Hox* gene expression have been identified in abnormal development and malignancy, and re-expression in many cancers such as pancreatic cancer[34], leukemia[35], and neuroblastoma[36]. In some tumors, altered expression of Hox genes directly drives tumorigenesis through escape from apoptosis[37], alterations to receptor signalling[38], epithelial-mesenchymal transition (EMT)[39] and tumour cell invasion[40]. Therefore, Hox gene expression is a prospective diagnostic marker and therapeutic target.

Hox genes encode a family of transcription factors, are usually conserved within metazoans, and are involved in generating pattern along the anterior-posterior body axis. Their involvement occurs during early embryogenesis collinearly with their arrangement on the chromosome[41,42]. In *C. elegans*, the *Hox* cluster includes six *Hox* genes arranged in three pairs spread out over 5 Mb of chromosome III. *Ceh-13*, *lin-39*, *mab-5*, and *egl-5*, are organized in a loose cluster[43-45], while the other two genes *nob-1* and *php-3* are located more than 1 Mb away on the same chromosome[46-48].

Kenyon *et al*[42] reported that lin-39, mab-5 and egl-5 are required for postembryonic development. Emmons[49] showed that *mab-5* and *egl-5* are involved in cell fate specifications in males, and Sternberg[50] reported that *lin-39* is mainly involved in vulval cell fates and selects the outcome of Ras signaling (Figure 2). Gener-

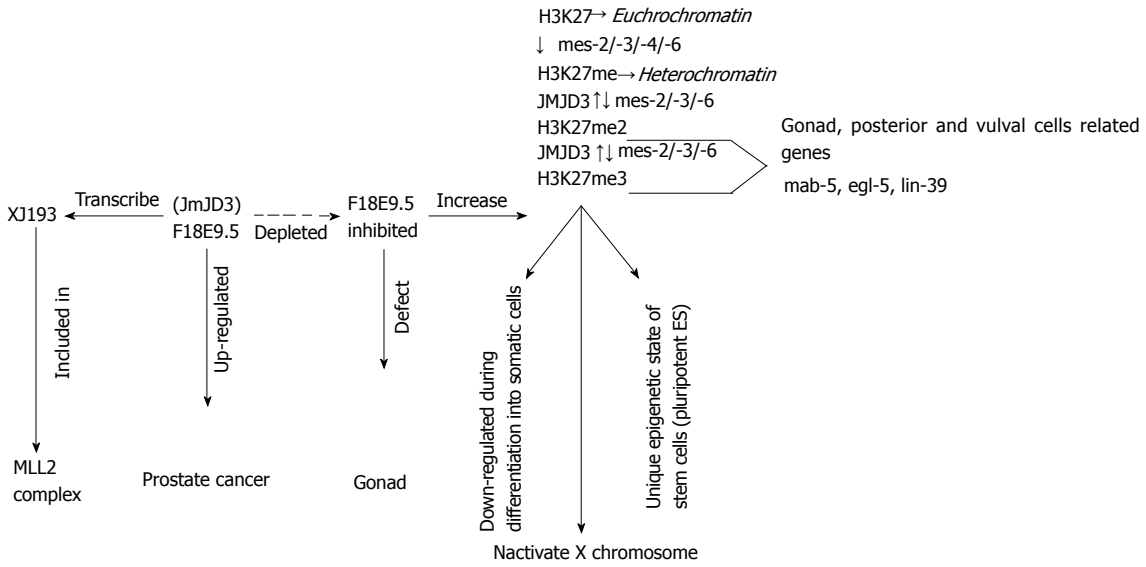

Figure 4 H3K27 modifications, Hox genes, and cellular development.

Table 4 Ubiquitin-conjugating enzymes in *Caenorhabditis elegans*: homologs and loss-of-function phenotypes

C. elegans	Peptide-conjugated	Human	Phenotypes
ubc-1	Ub	UBE2A; UBE2B	WT (RNAi)
ubc-2/let-70	Ub	UBE2D1/UBCH5A; UBE2D2/UBCH5B; UBE2D3/UBCH5C	embryonic arrest at pre-comma stage (RNAi)
ubc-3	Ub	CDC34; FLJ20419	WT (RNAi)
ubc-6	Ub	NCUBE1	WT (RNAi)
ubc-7	Ub	UBE2G1	WT (RNAi)
ubc-8	Ub	UBE2H	WT (RNAi)
ubc-9	SUMO	UBE2I	embryonic arrest post- gastrulation before muscle movement (RNAi)
ubc-12	NEDD-8 (Nedd8)	UBE2M	embryonic arrest at the comma stage (RNAi)
ubc-13	Ub	UBE2N; BAA93711	WT (RNAi)
ubc-14	Ub	UBE2G2	embryonic arrest post- gastrulation before muscle movement (RNAi)
ubc-15	Ub	NCUBE1	WT (RNAi)
ubc-18	Ub	UBE2L1; UBE2L3/UBCH7; UBE2L6	reduced growth rate and brood size (mut)
ubc-20	Ub	HIP2	impenetrant L3 and L4 larval arrest (RNAi)
ubc-21	Ub	HIP2	WT (RNAi)
ubc-22	Ub	UBE2L1; UBE2L3/UBCH7; UBE2L6	WT (RNAi)
ubc-23	Ub	HIP2	WT (RNAi)

ally, patterns of *Hox* gene expressions in *C. elegans* are controlled by cell lineage, which is completely different when compared to Drosophila or vertebrate systems[51]. However, as depicted in Figure 2, Wnt signaling pathways regulate some aspects of *Hox* gene expression during vulval development[52].

DNA methylation patterns are characterized by low tissue specificity when compared to other epigenetic information such as acetylation, phosphorylation, ubiquitylation, and ADP-ribosylation. These findings suggest that DNA methylation prediction is relatively easier to handle than other histone modifications[53]. In addition, *C. elegans* lacks cytosine methylation, and therefore it represents a potential candidate to explore for possible existence of an epigenetic code. This paper aims to investigate patterns of histone modifications in *C. elegans* that occur on Hox genes within a chromatin context. This investigation seeks to analyze the identified patterns and map histone modifications to the transcriptional status of specific *Hox* genes: histone code.

EXPLORING THE HISTONE CODE

Homeotic transformations that lead to body structure loss or duplication occur due to inappropriate expression of *Hox* genes. Thus, it is fundamental to identify a histone code that establishes a correlation between the histone modifications and a heritable histone code. In this work, only four *Hox* genes in *C. elegans* are investigated: *lin-39*, *mab-5*, *egl-5*, and *ceh-13*. It is known that each *C. elegans Hox* gene is expressed in restricted regions of multiple diverse tissues and lineally unrelated cells and defines the region specific differentiation characteristics[54].

Generally, *Hox* genes are globally repressed by the polycomb group (*PcG*) in mammals as well as in *C. elegans* (Figure 2). In addition, any mutations in *PcG* genes lead to ectopic *Hox* gene expression which will also in turn lead to posterior homeotic transformations in both Drosophila and vertebrates[55,56]. A further analysis of Drosophila *trxG/PcG* double mutants showed that activity of *TrxG/MLL* complexes is required to block *PcG*-mediated silencing of transcribed *Hox* genes. The *TrxG/MLL* complex that catalyzes the H3K4me3 is associated with active transcription[57,58]. Consequently, promoters of active genes develop to be enhanced with H3K4me3 modified nucleosomes. Similarly, in *C. elegans*, the *MLL2*

Table 5 Hox genes of the *Caenorhabditis elegans*: Homologs of humans

Gene product		Mammalian relative(s)	Molecular function
EGL-5	EGg-laying defects	Hox9-13 (all human posterior *Hox* genes)	Hox transcription factor. Upregulated by Ras signaling.
LIN-39	Abnormal cell LINeage	HoxB5	Hox transcription factor. Upregulated by Ras signaling.

complex plays the same role as in Drosophila (Figure 2).

Chromatins within mouse embryonic stem (mES) cells include M^2H3K4 and M^3H3K27 marks at *Hox* gene promoters, in which both repressive and activating chromatin modifications were referred to as "bivalent domains"[57]. These bivalent domains may lead *Hox* genes to an activation state. A challenging task is to determine how *Hox* genes become transcriptionally activated during ES cells differentiation or embryonic development.

Components of the *MLL2* complex in humans were shown to be initially recruited at the promoters of the most anterior *HOXA* and *HOXB* genes, with *H3K4* becoming tri-methylated[59]. The presence of UTX (Ubiquitously transcribed tetratricopeptide repeat, X chromosome, linked with histone dementhylation) with a simultaneous loss of *PRC2* and *H3K27me3* marks from the promoters resulted in a rapid activation of these genes. These findings suggest that UTX could be essential for activating *Hox* genes, since its loss of expression did lead to a strong decrease in *HOXB1* transcription. These findings are used later to support a particular hypothesis in conceiving the Histone code in *C. elegans*.

It has been reported in *C. elegans* that *mab-5* is expressed in the V5 and V6 cell lineages, which directs these cells to develop into rays. Further, *egl-5* is expressed only in the V6 lineage, which is required for the development and differentiation of V6 rays[60]. The normal development of the *C. elegans* male tails requires *SOP-2*, *MES-2/-3/-6* (*PcG* genes) and *lin-49/lin-59* (trithorax-related genes). As it is depicted in Figure 2, the misregulation of *mab-5* and *egl-5* is associated with the defects of ray development in *lin-49*, *lin-59*, *SOP-2*, and *MES*-mutants[61,62]. In *C. elegans* as well as in Drosophila, *PcG* proteins operate as transcription repressors, whereas trithorax proteins operate as transcription activators[63] .

Mutations in Hox genes lead to irregular patterns of programmed cell death. For example, in *C. elegans*, *mab-5* has been reported to be essential for the programmed cell deaths of two lineally related cells generated in the P11 and P12 lineages[64]. Further, Figure 2 shows that lin-39 was reported to control vulval cell development[65], and ceh-13 is required for development, and its ectopic expression during embryogenesis lead to embryonic lethality[66]. This latter is the orthologue of the Drosophila labial and the human *Hox1* genes.

Various parts of Figures 2-4 were constructed from exploring Wormbase (www.wormbase.org), which represents a major repository for *C. elegans* information. Figure 2 illustrates the fact that when *H3K4* is tri-methylated, it binds with the complex *lin-49/lin-59* which then expresses *mab-5* and *egl-5*. In normal cell development, *mab-5* and *lin-39* repress each other in turn. The loss of*H3K4me3* leads to X inactivation, whereas its increase occurs when rbr-2 (Jarid1 family) is inhibited, which then lead to a defect in vulval cells. However, it is not clear why *H3K4me3* binds with *JmJD2A*, and which proteins express or repress ceh-13, although its first expression is detected in the male tail from L3 until mid L4. In humans, components of the MLL2 complex were shown to be initially recruited at the promoters of the most anterior *Hox A* and *Hox B* genes, with *H3K4* becoming tri-methylated[59,67]. This finding suggests that *H3K4me3* could be involved in expressing *ceh-13*.

Figure 3 shows that all levels of methylated *H3K36* represent an activation mark and prevents transcriptional initiation downstream of the promoter region. This figure also shows a few correlative events. For example, *H3K36me3* expresses *lin-39*, whereas *H3K9me3* represses *lin-39*. The depletion of *JMJD2A* (H3K9/K36 demethylase) leads to an increase of *H3K9/K36me3* and to a *P53*-related apoptosis and an altered program of meiotic DSB repair. Like *H3K4me3*, *H3K9me3* has also been observed to bind with *JMJD2A*. However, no clue is available to comprehend the purpose of this binding.

Figure 4 illustrates the importance of H3K27me3 as it represents a unique epigenetic state of pluripotent ES cells, and it is mainly down-regulated during differentiation into somatic cells. Generally, all forms of methylated *H3K27* correspond to inactivation marks. However, *H3K27me3* is specifically involved in inactivating the X chromosome as well as *mab-5*, *egl-5*, and *lin-39*. The depletion of *F18E9.5* (member of JmJD3 family that demethylates *H3K27me3*) causes defects in gonadogenesis, whereas its up-regulation has been detected in prostate cancer[68] .

Figure 3 shows that the HMT met-2 catalyze H3K9 mono-, di- and tri-methylation in constitutive heterochromatin. The methylation of *H3K9* binds with the chromodomain of *hpl-2* in order to establish and maintain the heterochromatin structure.

Polycomb and trithorax groups are involved in maintaining the epigenetic code and the cell identity[69]. Polycomb complexes are found in closed chromatin structures and are thus involved in gene silencing, whereas trithorax complexes are found in open chromatin structure and are involved in maintaining active genes. Polycomb and trithorax groups are considered as HMTs and play a role in epigenetic inheritance[70,71]. Figure 4 depicts the mono-, di-, and tri- methylation of *H3K27* by the polycomb MES-2/-3/-6 proteins. In addition, it also shows that the di- and tri-methylation of *H3K27* are involved in repressing *mab-5*, *egl-5*, and *lin-39*. Further, Figure 2 shows that the trithorax complex *lin-49/lin-59* binds with *H3K4me3* and then activates *mab-5* and *egl-5*.

Figures 2-4 illustrate various paths that lead to expression and repression of *Hox* genes in *C. elegans*, and eventually help to describe the histone code: (1) *H3K4me3* is involved in activating *mab-5* and *egl-5*; (2)

H3K36me3 plays a role in activating *lin-39*; (3) *H3K9me3* is known in repressing *lin-39*; (4) *H3K27me2* and *H3K27me3* both repress *mab-5*, *egl-5*, and *lin-39*; and (5) although it is known that a high level of *pop-1* represses *ceh-13* and a low level of *pop-1* expresses it, nonetheless it is worthwhile investigating whether any histone modifications are involved in expressing or repressing it. Presently, evidential data only indicates that *H3K4me3* may perhaps express it. However experimental work is needed to support such a claim.

Histone acetylation is not yet demonstrated to be involved in epigenetic memory, since it is mainly a dynamic modification and is maintained by the ongoing activity of HATs and HDACs[21]. In addition, histone demethylase removes a methyl group from a particular histone tail. For example, *rbr-2* (Jarid1 family) demethylates *H3* at lysine 4 (Figure 2); JMJD2A protein demethylates *H3* at lysine 36 (Figure 2); and *F18E9.5* (*JmJD3* family) removes the tri-methyl group from *H3K27* (Figure 4). Correspondingly, other post-translationally modifications including phosphorylation, ubiquitylation, sumoylation, and ADP-ribosylation have not been shown to have an important role in epigenetic memory. Nevertheless, HATs and HDACs have been used in therapeutic targets in several diseases including cancer[72-75].

CONCLUSION

Histone modifications are clearly conserved within metazoans and correspond to a very ancient form of basal genetic regulation. Generally, each individual histone modification has the same biological effect in various organisms. For example, methylation of *H3K4* represents an activation mark in both humans and *C. elegans*. Evidently, the epigenetic code identified as the heritable transcriptionally states will contribute in biomedical research and particularly in epigenetic therapy. In addition, epigenetic regulation is shown to have a role in mental disorders, autoimmune diseases and many other complex diseases[76].

A number of silenced tumor suppressor genes are shown to be lost due to epigenetic deactivation rather than sequence damages, although epigenetic changes co-operate with genetic changes to initiate the development of a cancer since they are mitotically heritable[77,78]. Further, epigenetic irregularities are pharmacologically reversible as opposed to genomic damage[79]. This fact provides an incentive for the research community to devote more efforts to epigenetic therapy.

There is an on-going quest to discover drugs for diseases with genetic defects like cancer[80-83]. The purpose of investigating the histone code is to uncover the power of the epigenetic code and its use in drug design and discovery. Understanding the epigenetic machinery of the *Hox* genes and their cofactors could enable new targets for future therapies. As the investigation on Hox genes unravels, more translation to clinical application is expected. *Hox* genes have been used as biomarkers such as *HoxA9*[84], MLL translocation[85] and NUP98 fusions[86] in leukemias. In breast cancer, other groups have investigated the developed of a two-gene test using qRT-PCR to determine the ration of *HoxB13* expression to IL17RB expression that leads to predict the tumor recurrence in patients with eR-postive tumors taking tamoxifen[87,88].

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