Annals of Anatomy 192 (2010) 261–274

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Epigenetic control of *Hox* genes during neurogenesis, development, and disease

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ARTICLE INFO

Article history:
Received 19 July 2010
Accepted 20 July 2010

Keywords:
Hox genes
Epigenetics
Neurogenesis
Chromatin structure

SUMMARY

The process of mammalian development is established through multiple complex molecular pathways acting in harmony at the genomic, proteomic, and epigenomic levels. The outcome is profoundly influenced by the role of epigenetics through transcriptional regulation of key developmental genes. Epigenetics refer to changes in gene expression that are inherited through mechanisms other than the underlying DNA sequence, which control cellular morphology and identity. It is currently well accepted that epigenetics play central roles in regulating mammalian development and cellular differentiation by dictating cell fate decisions *via* regulation of specific genes. Among these genes are the *Hox* family members, which are master regulators of embryonic development and stem cell differentiation and their mis-regulation leads to human disease and cancer. The *Hox* gene discovery led to the establishment of a fundamental role for basic genetics in development. *Hox* genes encode for highly conserved transcription factors from flies to humans that organize the anterior–posterior body axis during embryogenesis. *Hox* gene expression during development is tightly regulated in a spatiotemporal manner, partly by chromatin structure and epigenetic modifications. Here, we review the impact of different epigenetic mechanisms in development and stem cell differentiation with a clear focus on the regulation of *Hox* genes.

1. Introduction

The process of mammalian development is established through multiple, complex, yet precisely controlled, molecular events at different genetic and epigenetic layers. Epigenetics are heritable changes beyond the DNA sequence that profoundly regulate embryonic development, stem cell differentiation, and cellular morphology with high impact in human disease. The epigenetic mechanisms which control cellular identities include: histone modifications, DNA methylation, chromatin condensation, and small RNAs. Epigenetic modifications dictate cellular identity through transcriptional regulation of key developmental genes resulting in specific cell fates. Epigenetics are reversible and mainly exist within the chromatin structure.

During differentiation of embryonic stem (ES) cells, the chromatin undergoes a global reorganization. This is associated with extensive genomic and epigenomic alterations leading to dramatic morphological changes. In ES cells, actively transcribed genes (e.g. *Oct4*, *Sox2* or *Nanog*, highly expressed in self-renewing ES cells) are characterized by histone acetylation and active chromatin marks (Lee et al., 2004; Meshorer et al., 2006). However, developmentally important genes that are inactive in ES cells possess both "active" and "inactive" histone marks such as histone H3 lysine 4 methylation (H3K4me) and histone H3 lysine 27 methylation (H3K27me), respectively. This group of genes carry the so-called "bivalent chromatin" marks (Bernstein et al., 2006), and are not expressed in self-renewing ES cells, but are poised for activation. Among them are many members of the homeobox (HB)-containing genes.

The homeobox is a 180 bp DNA sequence, which encodes for a 60 amino acid DNA-binding domain called the homeodomain (HD). The HB was discovered in *Drosophila* over 25 years ago as a conserved DNA sequence shared by developmentally important genes called the "homeotic genes", known presently as "*Hox* genes" (Gehring and Hiromi, 1986; McGinnis et al., 1984). Since then, many HB-containing genes and families have been identified in higher eukaryotes based on the conservation of their HB (Cantile et al., 2008; Duverger and Morasso, 2008). These genes have been divided into two classes; class I clustered HB genes (*Hox*), and class II dispersed non-*Hox* genes (Abdel-Fattah et al., 2006). Eight *Hox* genes exist in the fly and 39 such genes are present in mammals, arranged within four different clusters A to D on different chromosomes and organized into 13 paralog groups. *Hox* genes encode for highly conserved transcription factors with key roles in normal development (Dickson et al., 2009). By controlling downstream targets, HOX proteins tightly regulate the cellular and positional

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doi:10.1016/j.aanat.2010.07.009

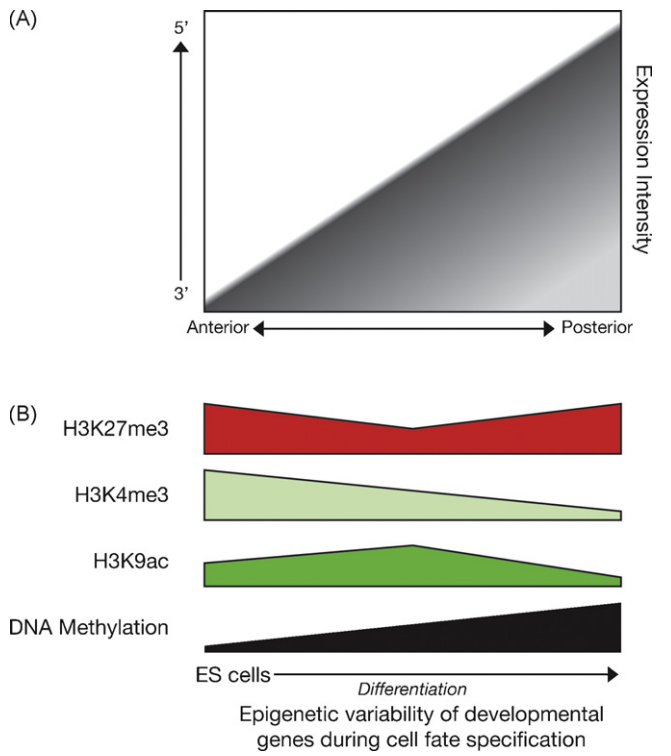

Fig. 1. Collinear *Hox* gene expression during development and epigenetic diversity during stem cell differentiation. (A) A generalized diagrammatic representation of 3′ to 5′ collinear *Hox* gene expression along the anterior–posterior axes is shown. The expression intensity is shown by the intensity of the gray color. (B) During ES cell differentiation, a global change in histone marks and DNA methylation is observed (Delcuve et al., 2009; Meissner et al., 2008; Mikkelsen et al., 2007).

identity of specific cell types along the AP axis of a developing embryo.

Hox genes are further known for their role in central nervous system development, where they play important roles in cellular identity, growth, differentiation, and cellular interactions with their environment (Abdel-Fattah et al., 2006; Lovegrove et al., 2006; Norris et al., 2009). *Hox* genes operate in a region-specific manner and their expression is tightly regulated in a spatially and temporally defined order, controlled in part by chromosome modifications and epigenetic changes (Soshnikova and Duboule, 2009; Vasanthi and Mishra, 2008). HOX proteins exert their regulatory role through specific targets that are involved in organogenesis, cell differentiation, cell adhesion and migration, cell cycle and apoptosis (Chuai and Weijer, 2009; Falaschi et al., 2010; Serpente et al., 2005; Stobe et al., 2009; van den Akker et al., 2010). HOX proteins bind to DNA with different cofactors, affecting their binding activity, sensitivity, and specificity (Huang et al., 2005). Through these interactions with different partners, HOX may activate or repress the expression of their targets. Furthermore, there are auto- and cross-regulatory interactions between *Hox* genes and their cofactors (Kobrossy et al., 2006; Svingen and Tonissen, 2006). The expression pattern of *Hox* genes is reflected in their chromosomal location, highlighting the importance of chromatin remodelling and chromosomal territories in the functional aspects of *Hox* gene expression. Genes located at the 3′ region of each *Hox* cluster are activated earlier and more anteriorly compared to the more 5′ genes which are expressed later and more posteriorly, a phenomenon referred to as "collinearity" (Fig. 1A) (Cantile et al., 2008; Rastegar et al., 2004). In addition to their role in AP patterning, *Hox* genes are also involved in different types of human disease and cancer including leukemia (Bijl et al., 2008), breast cancer (Raman et al., 2000) and brain tumors (Buccoliero et al., 2008).

In this review, we will summarize the epigenetic regulation of *Hox* genes, focusing on their implications in normal development and neurogenesis. First, we will present an overview on recent findings about epigenetic control mechanisms and the role of epigenetics in development and stem cell characteristics. Next, we will discuss our current knowledge about epigenetic mechanisms of *Hox* gene expression and silencing, with an emphasis on *Hox* gene participation in cellular differentiation. Finally, we will review *Hox*-related human disorders and the value of *Hox* genes for diagnosis and stem cell-based therapeutic strategies.

2. Epigenetics, development and stem cell neurogenesis

2.1. Epigenetic mechanisms work in harmony at different levels

Epigenetics are heritable changes controlling gene expression and cellular morphology through mechanisms other than the underlying DNA sequence. Epigenetic modifications are reversible and are mainly embedded within the chromatin structure and influence cellular response to environmental cues, dictating cellular identity. In eukaryotes, the chromatin is made of fundamental repeating units called the "nucleosomes". A nucleosome is a stretch of 146 bp DNA wrapped around a histone octamer [two H2A–H2B dimers and a $(H3\text{-}H4)_2$ tetramer], associated with one linker histone H1 (Olins and Olins, 1974) (Fig. 2). The epigenetic mechanisms working in concert to control gene regulation and cellular morphology include: histone modifications, DNA methylation, chromatin condensation and small RNAs. Chromatin structure and nucleosome distribution along the genomic loci can regulate gene expression by modulating DNA accessibility (Radman-Livaja and Rando, 2009). In humans, it has been shown that promoter regions have relatively low nucleosome concentrations compared to other genomic regions. However, the gene body regions contain a higher density of nucleosomes and more frequently display fixed nucleosome patterns (Kharchenko et al., 2008). While an explanation behind nucleosome positioning is still under investigation, there is increasing evidence for variation in positioning and functional consequences beyond transcription, including mRNA splicing (Andersson et al., 2009).

Post-translational histone modifications alter the interaction of N-terminal histone tails with the negatively charged DNA phosphate backbone. Table 1 represents different histone modifications and the corresponding chromatin modifiers. Variability in the architecture of the histone octamer and the DNA–chromosome interactions mediates gene expression *via* alteration at the condensed state of chromatin (Singh et al., 2009). Histone acetyl transferases (HAT) and histone deacetylases (HDAC) add and remove acetyl groups from lysine residues in the histone tails to relax or condense chromatin, respectively (Williams et al., 2006). Methylation of histone tails can activate or silence gene expression based on the lysine residues which have been marked (Munshi et al., 2009). Other post-translational modifications include variations of uni-, di- and tri-methylation, ubiquitylation, phosphorylation, sumoylation, and proline isomerisation (Kouzarides, 2007) (Fig. 2).

DNA methylation occurs at the 5′-CpG-3′ bases at the cytosine residue (Bird, 1980) (Fig. 2). At the promoter regions, DNA methylation can prevent transcription factor binding resulting in inhibition of active transcription (Voo et al., 2000), or promote binding of methyl binding domain (MBD) proteins, leading to gene suppression (Bogdanovic and Veenstra, 2009). DNA methylation is involved in transcriptional gene silencing affecting many developmental processes including X chromosome inactivation, DNA imprinting, genomic stability and chromatin structure (Delcuve et al., 2009; Feil, 2009; Li, 2002). Aberrant DNA methylation leads to