Epigenetic regulators and histone modification

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Abstract

Epigenetic inheritance is a key element in the adaptation of organisms to a rapidly changing environment without stably changing their DNA sequence. The necessary changes in its gene expression profiles are frequently associated with variations in chromatin structure. The conformation of chromatin is profoundly influenced by the post-translational modification of the histone proteins, the incorporation of histone variants, the activity of nucleosome remodelling factors and the association of non-histone chromatin proteins. Although the hierarchy of these factors is still not fully understood, genetic experiments suggest that histone-modifying enzymes play a major causal role in setting up a particular chromatin structure. In this article, the recent progress that was made to understand the molecular mechanisms of the targeting and regulation of histone modifiers and its implication for epigenetic inheritance are reviewed.

Keywords: chromatin; post-translational modifications; epigenetics; methylation

The term ‘epigenetics’ literally means ‘besides genetics’ and is commonly used to describe heritable alterations of phenotypic traits that are not based on changes in DNA sequence. In the literature it is used to explain many aspects of the transmission of genetic information ranging from the mitotically stable silencing of whole chromosomes [1–3] over the tissue specific maintenance of gene activity [4–6] to the heritable affect of nutrition on gene expression patterns [7, 8]. What all those divergent fields have in common is that a particular gene expression pattern is established by extrinsic or intrinsic factors, and is subsequently maintained through several cell divisions and sometimes even through meiosis making it an important evolutionary driver.

Frequently (but not always [9, 10]), these epigenetic changes are associated with stable changes in chromatins structure. The conformational changes of chromatin structure have been mostly studied using their differential accessibility to nucleases or RNA polymerases [11–14] and by various microscopic techniques [15]. The detailed molecular structure of chromatin in the eukaryotic nucleus, however, is still enigmatic. Over the last few years considerable progress has been made to characterize the structure of chromatin fibres in the test tube [16–19], suggesting that the intranucleosomal interactions of the histone tails have a substantial impact on the folding of higher order chromatin structure.

The N-terminal tails of the four core histones do not contribute to the formation of the nucleosomal core particle in vitro, and do not adopt a defined structure in the crystal of a mononucleosome [20]. However, the amino acid sequence of these N-terminal tails is highly evolutionary conserved. This extraordinary degree of preservation points to a strong selective force to maintain the sequence of the N-termini. One possible explanation for this conservation is the fact that the tails carry multiple post-translational modifications. The modifications in turn can then substantially influence chromatin structure.

HISTONE MODIFICATIONS

Studies on mononucleosomes as well as on nucleosomal arrays show for example that the acetylation of lysines within the tails facilitates the binding of transcription factors [21, 22], and disrupts the folding...
of nucleosomal fibres [23, 24]. Deletion of the N-termini of H3 and H4 is lethal in yeast [25], and the mutation of individual lysine residues leads to changes in gene expression patterns [26] that are enhanced when multiple lysines are mutated. In this study, the contribution of an individual lysine acetylation to establish a specific gene expression pattern is limited. Therefore, the findings do not support a model in which a complex combinatorial code is involved in setting up particular gene expression patterns. This interpretation is supported by a high resolution mapping, of modification patterns, of yeast mononucleosomes [27] that shows a rather low complexity of different modification patterns. However, the situation may be different in higher eukaryotes. Studies on polytene chromosomes of Drosophila melanogaster show a clear enrichment of H4K16ac on the male hyperactive X chromosome [28] and an enrichment of H4K12ac in inactive regions [28].

Although histone acetylation seems to be a good candidate to mark specific regions for transcriptional activation, because of its direct effect on chromatin structure, it is hard to reconcile the presence of this rather transient modification \( t_{1/2} = 3–30 \text{ min} [29] \) with the establishment of stable gene expression patterns that can be transmitted through cell divisions. In fact, no acetyltransferase has been isolated so far as genetic modifier in screens using stable gene expression patterns as a readout system. Acetylation does, however, have an impact on the establishment of stable gene expression patterns as a transient pulse of histone acetylation can switch on the expression of a transgene in Drosophila embryos, whose expression state can then be maintained over several cell generations [30]. This effect is dependent on the presence of a specific DNA element, within the transgenic construct, and a set of transcriptional regulators that play an important role in the maintenance of the tissue specific homeobox gene expression. The DNA elements are frequently called PREs or trithorax response elements (TREs) for polycomb and trithorax response elements or cellular memory modules (CMMs) [6, 31, 32]. Those short regions, on the DNA, are critical for establishing specialized chromatin structures that either repress or activate the neighbouring transcription units in a heritable manner. A heterogeneous set of chromatin-associated proteins is required to maintain this state either in a repressed state [the polycomb group (Pc-G) factors] or an activated state [the trithorax group (Trx-G)]. Both groups contain enzymes that are able to specifically modify the histone tails. However, in contrast to the acetyltransferases that generate modifications with a high turnover rate, the histone modifying enzymes that are found in the Pc-or Trx-G have mainly histone methyltransferase (HMT) activity. HMTases methylate lysine residues within the histone tails (arginine residues are methylated by enzymes that belong to the PRMT family [33], and can add up to three methyl groups per individual lysine. The slow turnover rate of methylated lysines [34] makes this modification a perfect candidate for maintaining a stable chromatin state through several cell divisions, and therefore, a key player in epigenetic inheritance. For a long time histone methylation has been considered irreversible, being reversed only by the exchange of methylated histones with unmethylated ones [35] or by proteolytic cleavage of the tail [36, 37]. However, more recently several histone demethylases have been shown to actively remove methyl groups methylated histones through an oxidative mechanism [38–40].

**TARGETING OF MODIFIERS**

In order to serve as a mark that has the potential to distinguish different regions of the genome the modifications have to be directed to specific loci. There are several ways of targeting modifying enzymes to their site of action (Figure 1). The most intuitive one is probably the targeting by an interaction with sequence specific transcription factors. This leads to a more localized modification that can in extreme cases be limited to a single nucleosome at the promoter [41] of a given gene. Such a targeting mechanism has been shown for acetyltransferases [42–44], deacetylases [45, 46], methyltransferases [47–49] and also more recently demethylases [38, 40]. Besides this very specific action, histone-modifying enzymes have also been shown to interact with proteins that track the DNA such as the RNA polymerases [50–52] or the replication clamp proliferating cell nuclear antigen (PCNA) [53], which leads to a modification of larger genomic loci in the process.

More recently, a third mechanism of targeting histone-modifying activities has been proposed that involves the transcription of non-translated RNAs from the corresponding gene locus. The inactivation of the mammalian X-chromosome as well as the
hyperactivation of the male X-chromosome in dipterans for example are both crucially dependent on the presence of a RNA molecule, which in turn is important for the accumulation of specific modification marks on the whole chromosome [54–57]. Also the transcriptional repression of peri-centromeric DNA is dependent of the RNAi machinery that helps targeting the methylation of H3K9 to this chromosomal domain. Elegant experiments in Schizosaccharomyces pombe showed that the RNAi machinery is important for the establishment but not the maintenance of centromeric heterochromatin [58–60]. The subsequent biochemical characterization of the protein complex responsible for RNAi induced transcriptional silencing, the repeat induced transcriptional silencing (RITS) complex showed that it contains another chromo domain protein that is able to interact with H3K9me suggesting a very intricate relationship between dsRNA, histone methylation and heterochromatin formation [58, 61]. With the discovery of the RNAi mediated transcriptional silencing complex and the involvement of the RNAi machinery in the heterochromatin formation, many of the RNA molecules that are derived from intergenic transcription have gained considerable attention. Even in the already discussed classical epigenetically inherited expression profile of homeobox genes, the binding of a RNA molecule has been shown to play a crucial role in the targeting of a HMT to their site of action [62]. In this case, the HMT Ash1 that belongs to the Trx-G requires active transcription from the TRE to be targeted to a specific area. Ash1 itself binds to RNA, which provides a good example for the targeting of a functionally active histone-modifying enzyme. These selected examples already show that the histone modifying enzymes can be selectively targeted to distinct loci within the genome by various mechanisms, ranging from the ability to modify small regions of just a few nucleosomes

Figure 1: Targeting mechanisms for histone modifications. Histone modification patterns can be established over defined chromosomal regions by multiple mechanisms. All targeting systems require a targeting molecule that could bind a DNA sequence like many sequence-specific transcription factors (a). Recognize a specific modification (b). Or a non-translated RNA molecule (c). The targeting molecule can then selectively interact with a specific histone modifier to set up a defined histone modification pattern.
to whole chromosomes such as the inactive female X-chromosome in mammals or the hyperactive male X-chromosome in fruit flies.

**RECOGNITION OF MODIFICATIONS**

How are the modifications then translated into a defined chromatin structure, which can then subsequently lead to an epigenetic change? In the case of histone acetylation, the modification in itself changes the physicochemical properties of the chromatin fibre such that an acetylated array adopts a more open conformation. However, histone methylation clearly does not have such an effect on chromatin folding. What was found however, is that the various modifications can be recognized by non-chromatin proteins that can either lead to a structural change in chromatin such as the heterochromatin protein 1 (HP1) that is thought to stabilize a condensed higher order structure [16], enzymes that can either mobilize nucleosomes [63, 64], modify the associated DNA [65] or further modify the histones [66–68]. The two classes of domains that are found to interact strongly with specific chromatin modifications can be categorized into the chromo-domain or tudor superfamily [69] that binds preferentially methylated lysine residues and the bromo domain family that recognizes acetylated lysines [70]. However, the ability to recognize specific marks does not seem to be exclusive to these domains as it has recently been shown that another domain that is commonly found in chromatin associated factors, the WD40 domain also possesses the ability to interact selectively with methylated lysine residues [71]. In this respect it is also important to mention that many chromatin associated factors contain several domains that can potentially interact with modified chromatin. This may suggest that these factors can indeed read a particular modification pattern on histones by binding to a complex combination of modification. However, the affinities of the factors to the modified tails in vitro are relatively weak, therefore, the contribution of a modification to a specific association with chromatin is probably not the only one [72, 73].

Especially, the discoveries of protein domains that are able to recognize specific modifications or combinations thereof make the histone code hypothesis are very attractive model to explain epigenetic inheritance [74]. The hypothesis was initially proposed by Turners and colleagues [75], and suggests that different histone modifications code for specific chromatin conformations. These different modifications could act in a combinatorial manner and heritably alter gene expression without a change in the DNA sequence. As the nucleosomes immediately reassociate with the newly replicated DNA, the modification patterns can potentially be transmitted through several rounds of cell divisions and maintain the chromatin structure. Although this is a very appealing model, experiments in yeast do not support the idea of a complex combinatorial code but rather suggest a simple on–off switch with chromatin being either in an open or a closed conformation [26, 76]. It remains to be seen whether this is also true in the case of higher eukaryotes and more complex modifications.

In summary, one can conclude that our understanding of the exact way how particular modification patterns are established and maintained is still very preliminary. Genetic experiments such as screens for modifiers of position effect variegation or regulators of Hox-expression have led to the identification of a large number of factors that participate in the establishment of a defined functional state of the underlying chromatin. The next challenge will be the deciphering of the precise mechanisms that allow those factors to set up a specific structure. One key to do this will be the reconstitution of distinct chromatin structures in the test tube by sequentially adding all the necessary factors. The analysis of all the necessary and sufficient players to set up a defined structure in vitro should then allow us to develop and test a model of how epigenetic signals are set and interpreted in vivo.

**NOTE ADDED IN PROOF**

However, while the manuscript was under review, crystallographic studies using the WD40 domain of WDR5 showed that it is able to bind the H3 N-terminus irrespective of its methylation state. Instead of ‘reading’ a modification state the domain has how been suggested to present the tail to the modifying enzyme [77, 78].

**Key Points**

- Post-translational modifications on Histones play an important role for epigenetic inheritance.
- Methylation of lysines plays a key role in setting up patterns of PTMs.
- The targeting of modifying enzymes is mediated by diverse mechanisms. Certain chromatin proteins can recognize specific modifications.
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References
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