Lecture No 3. Chromatin Structure and Function II (continued)

As a follow up to the last lecture we will discuss here two more histone posttranslational modifications; histon methylation and phosphorylation.

2- Histone methylation

Another very important histone posttranscriptional modification is the addition of methyle groups to the N-termini of the core histones. This process is called histone methylation and is carried out by a group of enzymes called histone methyl transferases. Histone methylation always occurs in specific lysine and arginine residues (Quina, *etal*., 2006). The specific histone residue could be mono, di or tri-methylated (Fig1) (Rice and Allice, 2001). In this lecture we will talk about forms of histone methylation, histone methyltransferases, the consequences of histone methylation and its effect on gene expression.

First, Histone methylation has a larger half-life, frequency in a time-scale of hours, and are therefore considered as stable modifications that might contribute to epigenetic ‘‘memory" by fixing the chromatin organization in a heritable manner (Quina, *etal*., 2006; Rice and Allice, 2001).

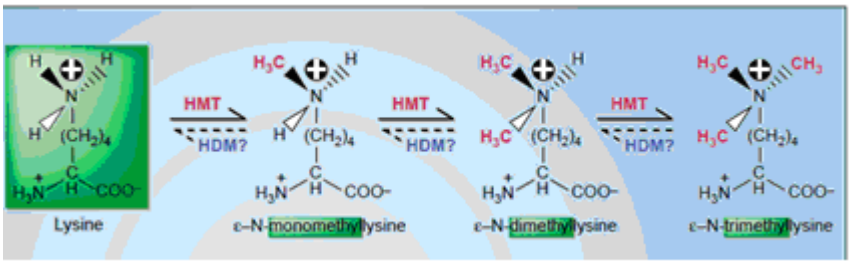

Fig1: Histone methylation (Rice and Allice, 2001).

Forms of histone methylation

Arginine methylation is a common posttranslational modification (PTM). This type of PTM occurs on both nuclear and cytoplasmic proteins, and is particularly abundant on shuttling proteins. Importantly, arginine methylation of histone tails can promote or prevent the docking of key transcriptional effector molecules, thus playing a central role in the orchestration of the histone code. Arginine methylation in the tails of histones can be monomethylarginines (MMA), or dimethylarginines. There are two forms of the dimethylarginines; asymmetric dimethylarginines (ADMA), and symmetric dimethylarginines (SDMA). The MMA form of arginine is generally regarded as an intermediate on its way to the dimethylated state and is not depicted (Fig 2) **(**Lorenzo and Bedford, 2011**).** In contrast to histone arginine methylation which is mainly linked to transcriptional activation, Lysine modification could be associated to either activation or repression of the transcription, depending on the site and status. Lysine could be mono, di-, or trimethylated.

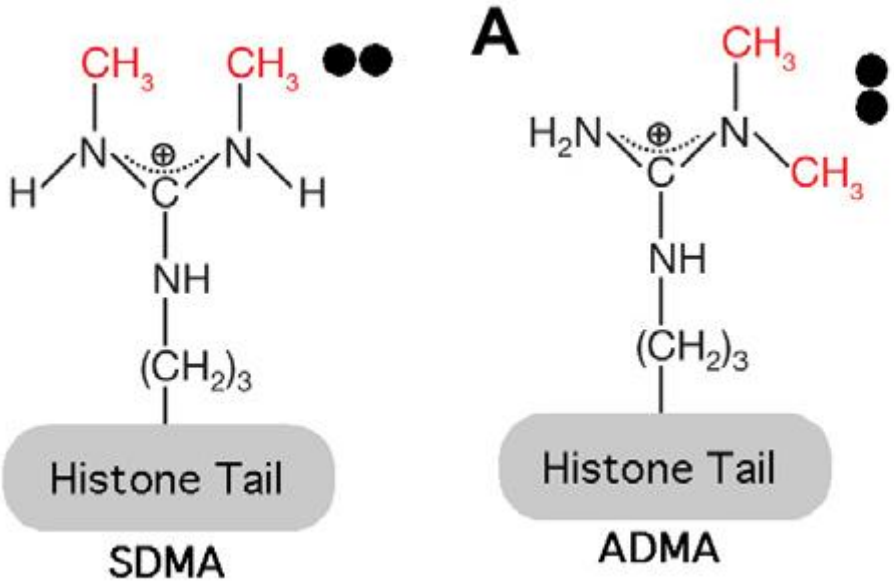

Fig 2: Forms of Arginine methylation (Lorenzo and Bedford, 2011**)**

Histone Methyltransferases

Both lysine and arginine residues from histones H3 and H4 can be methylated by enzymes that belong to three different protein families. The PRMT protein family uses arginines as a substrate, while the SET domain-containing protein family and the Dot1/DOT1L proteins are specific for lysines. Furthermore the SET domain-containing histone lysine methyltransferases are grouped into seven families; (i) The SET1 family is characterized by the presence of a SET domain followed by a Post-SET motif at the C-terminus of the protein, (ii) The SUV39 family includes HKMTs harbouring a SET domain flanked by Pre-SET and Post-SET motifs, (iii) The SET domain of the HKMTs from the SET2 family is flanked by AWS and Post-SET motifs, (iv) HKMTs from the EZH family harbour the SET domain at the C-terminus of the protein as well as SANT motifs, (v) The members of the SMYD family contain a MYND-type zinc finger preceding the SET domain, (vi) The proteins of the PRDM family possess striking amino acid changes in a highly conserved motif (NHSCxPN, where x is a non-conserved amino acid) within the SET domain. However, two of the members have been shown to have an HKMT activity specific for H3K4 (PRDM9) or H3K9 (PRDM2). Moreover, most of the members contain a number of C2H2-type zinc fingers, (vii) a group of SET domain-containing proteins falls in none of these families (Fig 3) (Vo¨lkel and Angrand, 2007).

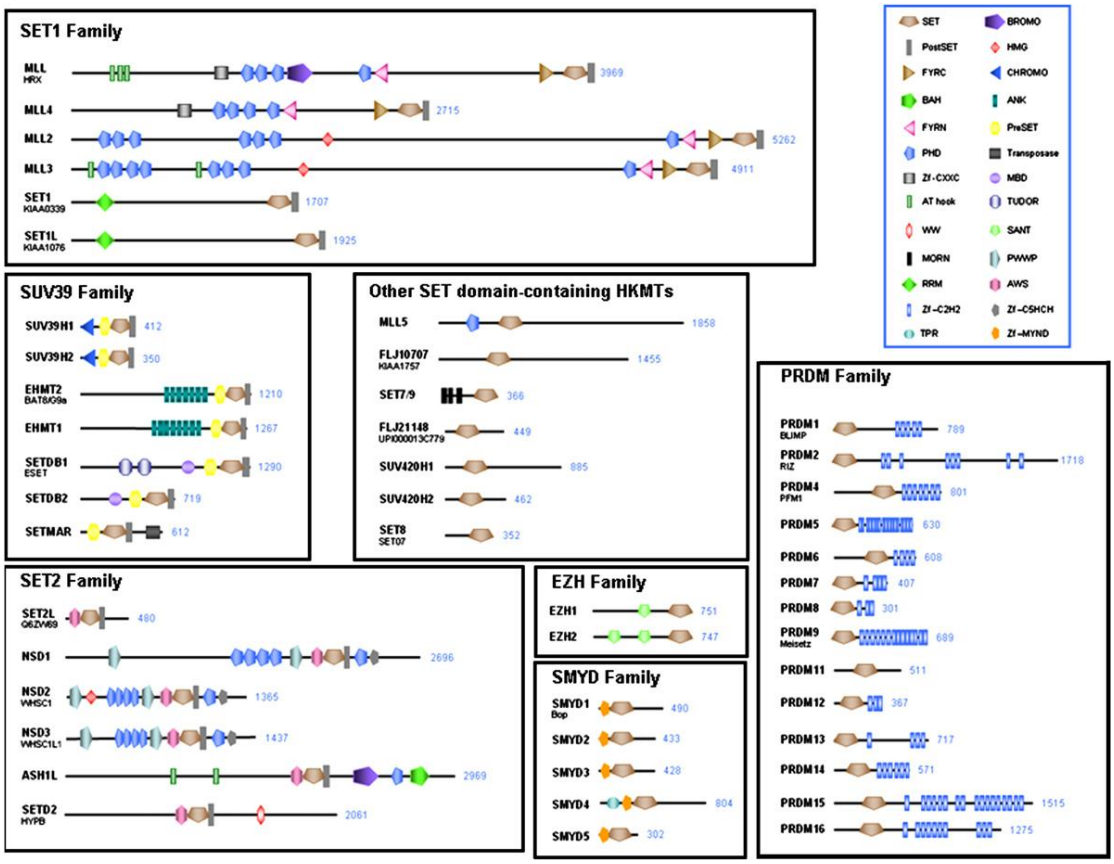

Fig 3: Structural diagram for Histone methyltransferases (Vo¨lkel and Angrand, 2007).

Consequences of Histone methyltransferases and the effect on gene regulation

Histone methylation on both arginine and lysines has a strong control upon gene expression regulation. This control, whether activation or repression for gene expression, differs based on the form and the status of the methylation**.** Chromatin-immunoprecipitation (ChIP) experiments have shown that expressed genes are methylated at lysine 4 (H3K4), lysine 36 (H3K36) and lysine 79 (H3K79) of histone H3 whereas methylation at lysine 9 (H3K9) and lysine 27 (H3K27) of histone H3 and methylation at lysine 20 of histone H4 (H4K20) are epigenetic marks of a repressed chromatin state at the chromosomal level.

Methylation of H3K9 and H4K20 is involved in the formation of heterochromatin. In a number of species and in a variety of cases, the SUV39 family of H3K9 HKMTs has been shown to be the major determinant of constitutive heterochromatin. Thus before we discuss the role of histone methylation in the formation of heterochromatin we will explain briefly the structure of heterochromatin.

Heterochromatin was initially defined as chromosomal regions that remain condensed throughout the cell cycle and are associated to specific domain such as centromeres, telomeres, and pericentric regions that are satellite-repeat-rich. In the first step of heterochromatin formation, the SUV39H1/2 HKMTs are targeted to repeat-rich sequences at pericentric heterochromatin. Because pericentric chromatin is also enriched in H3K27me1, and the SUV39H1/2 enzymes preferentially use H3K9me1 substrates, this initial step requires the activity of other H3K27 and H3K9 monomethylases. The SUV39H1/2 HKMTs produces the H3K9me3 epigenetic marks that will generate docking sites for the chromodomain-containing proteins HP1a and HP1b. Targeted HP1 molecules further recruit other SUV39H1/2 HKMTs that will favor the spreading of H3K9me3 methylations on one hand, and recruit the SUV420H1/2 HKMTs which in turn will tri-methylate H4K20 on the other hand. Since SUV420H1/2 di- and tri-methylate H4K20, the heterochromatin formation requires the activity of an additional H4K20 monomethylase. It is important to mention here that mutations in the Drosophila homolog Su(var)4-20 impair heterochromatin formation in fly. Consequently, constitutive heterochromatin formation depends on the interplay of several different HKMTs in addition to SUV39H1/2 and SUV420H1/2 (Fig. 4).

In contrast to histone acetylation, methylation of lysine residues does not alter the charge of the lysines. Histone methylation might generate docking sites for specific proteins that mediate downstream effects and chromatin organization. Whereas the bromodomain recognizes acetylated lysines, several different protein domains were shown to be involved in

methyl-lysine recognition: the chromodomain , the WD40 repeat, the Tudor domain, the MBT domain and the PHD finger. Proteins that contain chromodomains, WD40 repeats, Tudor domains, MBT domains or PHD fingers are recruited at specific methylated lysines and this interaction might be responsible for the specific biological outcomes, associated to precise methylation events (Vo¨lkel and Angrand, 2007).

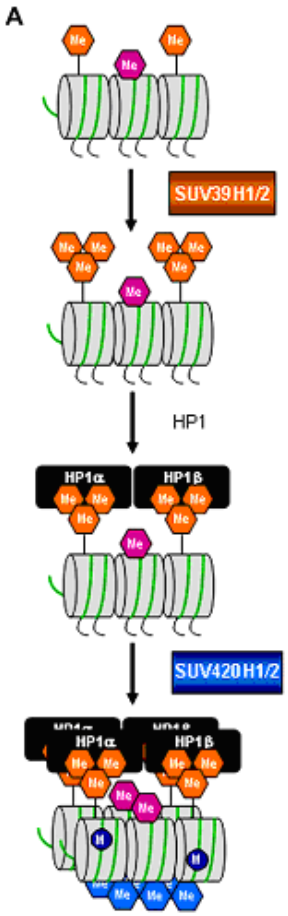

Fig 4: Formation of heterochromatin (Vo¨lkel and Angrand, 2007).

3- Histone Phosphorylation

Another Important histone modification is phosphorylation, which means the addition of phosphate groups to the hitone tails. It is not known till present how histone phosphorylation affects gene expression. One possibility is that the addition of negatively charged phosphate group to the N-terminal H3 tails may disrupt electrostatic interactions between the basic H3 tails and the negatively charged DNA backbone, and thereby increase the accessibility of the underlying genome to the nuclear factors. Thus histone phosphorylation, like acetylation, is associated with active gene expression. Interstingly, it has been found a mechanistic link between these two processes (acetylation and phosphorylation) where Histone acetyl transferases were shown to preferentially bind to phosphorylated histone tails at ser10.

Histone phosphorylation, however, is implicated in a wide range of cellular processes. It is involved in both chromatin relaxation upon receiving mitogenic signal (Fig 5A) and chromatin condensation upon receiving a cell cycle signal (Fig 5B). Based on the site of phosphorylation, the activity of the cell process is identified. For example, phosphorylation of H3 at serine 10 or 28 residues activates transcription and mitosis. On the other hand, phosphorylation of H2A.X is involved in DNA repair, while phosphorylation of H2B is involved in both DNA breaks and apoptosis (Fig 6) (Cheung, *et al*., 2000).

Fig 5A **Fig5B**

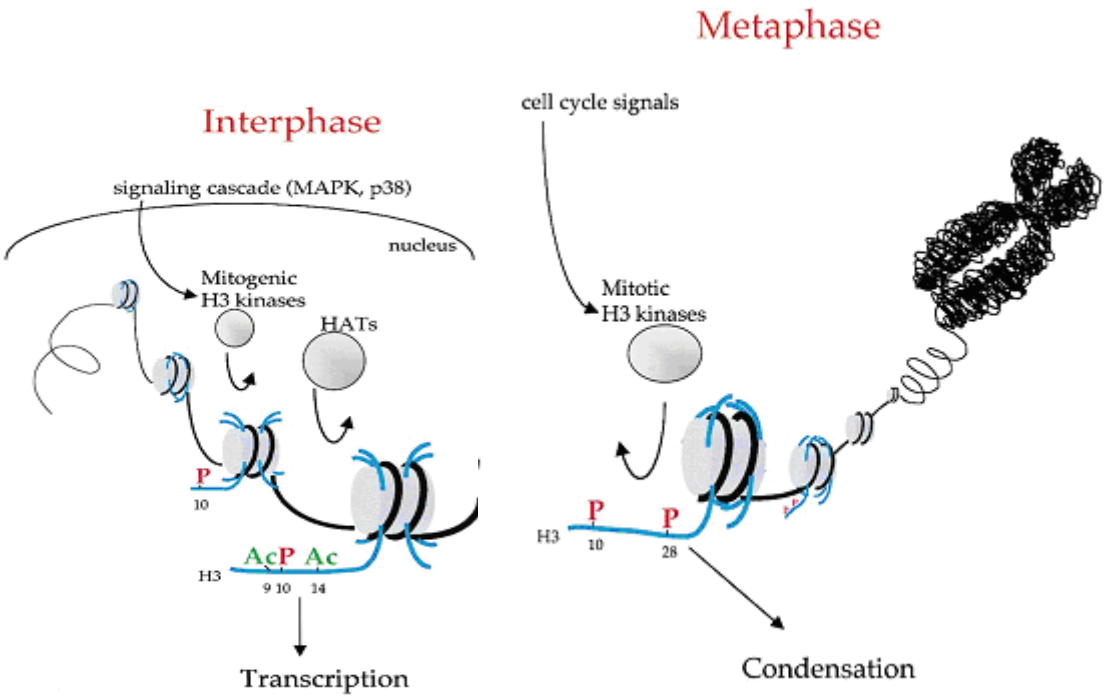

Fig 5: Histone phosphorylation is involved in cell division (Cheung, *et al*., 2000)**.**

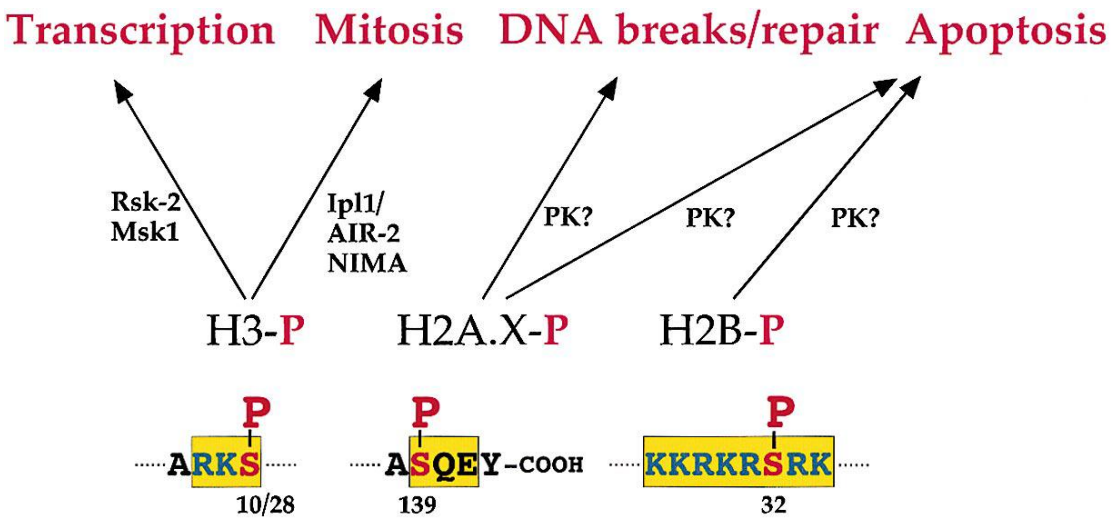

Fig 6: Multiple cellular processes are associated with Histone Phosphorylation (Cheung, *et al*., 2000)**.**

The cross talks between different Histone modifications

For the regulation of transcriptional activity it is particularly relevant the ‘‘dialogue’’ between methylated and acetylated marks and, indeed, the list of enzymes that potentially acetylate, deacetylate or methylate nucleosomal histones has been growing in the last years. In vitro studies suggest that histone acetyltransferases (HATs) and deacetylases (HDACs) can target different lysine residues within histones, while most histone methyltransferases (HMTs) have higher specificity for particular arginine or lysine residues.

Chromatin modulators possess at a set of conserved domains (including bromo and chromo domains) that catalyze or recognize histone modifications. These protein modules bind specifically to different lysine modifications and can thus act as starting transmission points of appropriate regulatory signals. Specifically, the bromo domain interacts selectively with acetylated lysines and is in general linked to transcriptional activity, whereas the chromo domain may work as a recognition module for methylated marks and is typically associated with gene silencing and assembly of heterochromatic domains. Additionally, within lysine modifications, the protein domains may be specific depending on the position of the residue in the histone. For instance, the chromo domain from HP1 is selective for H3-K9, and only poorly binds to H3 peptides with methylated lysine K4. Moreover, lysine residues may be mono-, di- or tri-methylated, adding even more complexity to the signalling cues generated by this mark. Not all methylated marks correlate with gene silencing, and some acetylated marks repress instead of activate transcription. For instance, H3-K4 methylation seems to constitute an euchromatic mark, and methylation of arginines in histones H3 and H4 synergistically lead to transcriptional activation. By contrast, acetylation of H4-K12 seems to reinforce a silent chromatin state (Quina, *et al*., 2006)

Histone modifications are interdependent and can favor or repress other modifications. In histone H3, phosphorylation of serine 10 inhibits methylation of K9, and may act in a synergistic manner with acetylations of K9 and K14, or methylation of K4. On the other hand, deacetylation of H3-K14 facilitates the subsequent methylation of K9 (Quina, *et al*., 2006)**.** A body of evidence showed that suv39h1 knockout murine cells suggests that methylation of H3 at lysine 9 can inhibit phosphorylation of ser10 at this histone tail. Also hence lysine 9 can be either methylated or acetylated, these two processes can be antagonistic to each other (Fig 7) (Cheung 2000).

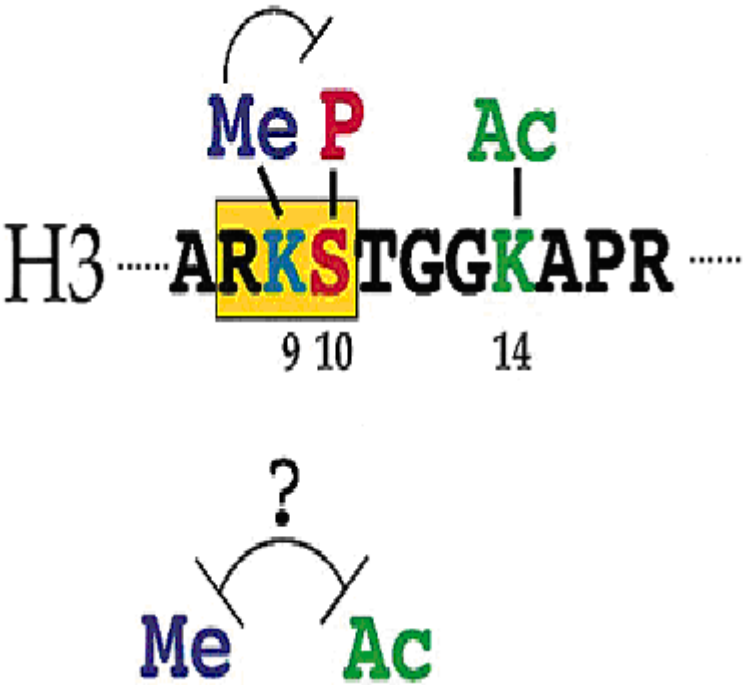

Fig 7: Interplay between different histone modifications (Cheung 2000)**.**

References

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3- Lorenzo, et al., (2011). Histone arginine methylation. FEBS Letters 585: 2024–203

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