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Transcriptional Control: An Activating Role for Arginine Methylation

Dispatch

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A rare histone modification, arginine methylation, has been linked to activation of hormone-responsive genes. Interestingly, methylation of a lysine residue in the same histone is present prior to hormone activation, but is excluded from the active loci.

Post-translational modification of histones plays a central role in the regulation of gene expression. Histone acetylation, in particular, is linked to gene activation, whereas deacetylation is linked to repression. Histone phosphorylation is connected not only to gene activation, but also to chromosome condensation and DNA repair. Histones are subject to ubiquitination and methylation as well, but the functions of these modifications are poorly understood. In general, histone modifications are thought to affect both the structure of chromatin and its interactions with non-histone regulatory factors. Recent studies have shown that the relatively rare histone modification of arginine methylation can play an important role in the transcriptional activation of certain genes.

Histone Methylation

Although histone methylation has long been observed in a number of organisms, only recently have specific roles for this modification come to light. The known sites for histone H3 methylation and the enzymes identified are summarized in Figure 1a. The most studied methylation event occurs on Lys9 of histone H3 and is mediated by members of the Su(var)3-9 family of methyltransferases. This family of methyltransferases was originally identified in *Drosophila* (Su(var)3-9), and homologs have been described in *S. pombe* (Clr4), mice (*Suv39h1 and Suv39h2*) and humans (SUV39H1). A characteristic feature of proteins in this family is the presence of a 'SET' domain. Methylation of residue Lys9 of histone H3 is strongly associated with gene silencing and the formation of heterochromatin [1,2]. This modification creates a binding site for heterochromatin-specific proteins, such as HP1. Interestingly, HP1 also binds to the methyltransferase Su(var)3–9, providing the possibility for feedback regulation as well as for the spread of the H3 Lys9 methylation and HP1 binding to neighboring nucleosomes. Histone H3 Lys9 methylation may also function in repression outside of heterochromatin, as SUV39H1 can cooperate with the Rb protein in repression of euchromatic genes [3].

Disruption of both mouse genes *Suv39h1* and *Suv39h2* causes severely reduced viability and chromosome instabilities which lead to increased tumor formation and spermatogenic failure [4]. The chromosomal instability in these mice highlights the importance of this histone modification in the establishment of heterochromatin, and moreover the importance of proper heterochromatin maintenance. Intriguingly, two antibodies specific for Lys9-methylated isoforms of histone H3 were used to characterize the double *Suv39h1-h2* mutant mice. One antibody exhibited high specificity towards pericentric heterochromatin and lost reactivity in the double null mice, as expected; the second antibody exhibited a more diffuse staining pattern that was not abolished. These results suggest that additional, unidentified histone methyltransferases can methylate this residue outside of pericentric heterochromatin, suggesting additional functions for histone H3 Lys9 methylation.

Histone H3 can also be methylated at Lys4, but the function of this modification is as yet poorly understood. This modification is associated with the transcriptionally active macronucleus of *Tetrahymena* [5]. In the fission yeast *Schizosaccharomyces pombe*, methyl-histone H4 Lys4 is found in euchromatic regions and appears in an exclusive, complementary pattern with methyl-histone H3 Lys9 [6]. A similar relationship is observed at the chicken β-globin locus, where methyl-histone H3 Lys4 is anticorrelated with methyl-histone H3 Lys9, but correlates well with histone H3 acetylation [7].

Interestingly, histone H3 Lys9 methylation has not been detected in the budding yeast *Saccharomyces cerevisiae*, which has very little heterochromatin, but histone H3 Lys4 methylation is abundant. No Su(var)3–9 family member is readily apparent in the *S. cerevisiae* genome, but up to six genes (*SET1-6*) encoding proteins with SET domains have been identified. Set1 methylates histone H3 at Lys4 *in vitro*, and both *SET1* and histone H3 Lys4 methylation are required for complete rDNA silencing [8]. It will be very interesting to determine whether *S. cerevisiae* uses Lys4 in gene silencing and the formation of heterochromatin-like structures as other organisms use Lys9, or whether the methylation of each of these sites has multiple functions in gene activation and repression. Another family member, Set2, methylates histone H3 Lys36, and appears to have a repressive function *in vivo* [9].

Until recently, little was known about the role of arginine methylation of the histones. The significance of this modification has been questionable, because it occurs rarely and is present at very low abundance. However, the discovery of two histone arginine

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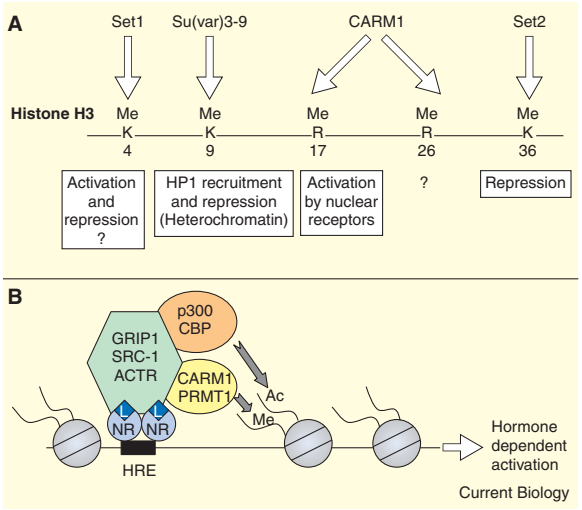

Figure 1.

(A) Methylation sites in the histone H3 tail, the enzymes identified to methylate these sites (top), and potential roles ascribed to the modified sites (bottom). (B) A schematic model for hormone-dependent transcriptional activation. The nuclear hormone receptor (NR) –ligand (L) complex binds to a hormone response element (HRE). The NR recruits coactivators (GRIP1, SRC-1, ACTR) which then recruit secondary coactivators (CBP, p300 and CARM1, PRMT1). CBP and p300 can acetylate all four core histone tails. PRMT1 and CARM1 methylate H4 and H3 tails, respectively. Although shown here on separate molecules, acetylation and methylation can occur within the same histone tail.

methyltransferases, CARM1 and PRMT1, as cofactors required for responses to nuclear receptor hormones provided an indication that histone arginine methylation does have an important role in transcriptional regulation [10].

Hormone-dependent Activation

Activation of transcription mediated by nuclear receptors is a complex, multistep process [11]. As illustrated in Figure 1b, ligand-bound nuclear receptors bind specific enhancer elements and recruit coactivators of the p160 family (SRC-1, GRIP1, ACTR). These co-activators recruit secondary coactivators including histone acetylases (p300, CBP) and arginine-specific protein methyltransferases (CARM1, PRMT1). Activation of hormone-responsive genes clearly requires chromatin remodeling and modification, and unraveling the co-factors and identifying the histone modifications involved is a major research focus.

In transient transfection assays with hormone-responsive reporter genes, multiple co-factors with chromatin modifying activities were found to function synergistically to achieve high levels of transcription. In the presence of a p160 co-activator, for example, CARM1 and p300 function together to yield a higher level of activation than can be achieved by either factor alone [12]. The same is true for CARM1 and PRMT1 [13]. Acetylation of multiple lysines within the core histones by p300 may act cooperatively with methylation of specific arginine residues in H3 by CARM1. Such cooperation appears to be the case for PRMT1. *In vitro*, PRMT1-methylated histone H4 is a better substrate for the histone acetyltransferase p300 and, conversely, the acetylated tail is a poor substrate for PRMT1 [14]. Even the two arginine methyltransferases involved in these responses have distinct histone targets that may each contribute gene activation. CARM1 methylates H3 Arg17 and Arg26 [15], while PRMT1 methylates histone H4 Arg3 [14,16].

One limitation of transient transfection experiments is the inefficient packaging of the reporter genes into chromatin. Several studies have documented the importance of chromatin for proper regulation by the nuclear receptors. For example, mouse mammary tumor virus (MMTV) reporters only respond properly to glucocorticoids when assembled into chromatin templates [17]. To avoid such problems, Hager and colleagues [18] have developed cell lines with large, tandemly integrated reporters carrying MMTV promoters. One cell line in particular is an exceptionally powerful tool, as the integrated MMTV array is large enough to be visualized by light microscopy after DNA staining. Immunocytofluorescence using antibodies to specific transcription factors or histone isoforms also allows visualization of active transcription, receptor binding and specific histone modification states.

As recently reported in *Current Biology* [19], Ma *et al.* took advantage of this powerful system to demonstrate the molecular synergy between GRIP1 and CARM1 in nuclear receptor responses. They showed that GRIP1 and CARM1 cooperate to enhance hormone-dependent activation of multiple tandemly integrated copies of a CAT reporter gene controlled by the MMTV promoter. Effects of specific mutations showed that the methyltransferase activity of CARM1 is necessary for its co-activator function. CARM1 and the CARM1-methylated form of H3 (methyl Arg17) associated with the MMTV promoter in a hormone-dependent manner. Conversely, methylation of histone H3 Lys4 was found to be excluded from the array upon hormone activation.

Dynamics of Histone Methylation: Is It Reversible?

An exciting implication of these results [19] is that histone methylation is dynamic. No histone demethylating activities have been discovered to date, questioning the reversibility of the modification. Some models suggest that proteolytic cleavage of the histone tails, or replacement of the entire methylated histone, is necessary to remove this mark from chromatin [20]. However, the presence of histone H3 Lys4 methylation in the absence of hormone treatment, the rapid exclusion of this modification upon hormone treatment and the concomitant gain of a new methylation mark a few amino acids downstream (at Arg17) strongly indicate that at least some methylation events are reversible. In this case, proteolysis of the H3 tail would have to be extremely limited to prevent removal of both Lys4 and Arg17. Replacement of the modified

histone — by reassembly of nucleosomes associated with the array — would also have to be very rapid. Using the system described by Ma *et al.* [19], time course studies of histone H3 methylation dynamics should be revealing as to the mechanism of the 'methylation exchange' and may provide a new tool in the search for the elusive histone demethylase (or proteolytic) activities.

The complex network of histone modifications that occur in nuclear receptor-mediated gene activation strongly support the existence of a 'histone code': the concept that the specific modification state of the histones plays crucial, specific regulatory roles and thus extends the information content of the genome past the DNA code [20]. The complexity of functions associated with the modifications within this single histone, H3, in nuclear hormone-dependent activation highlights the immense regulatory potential of chromatin. Each histone has multiple sites of modification, so thousands of combinations are possible. The challenge for the future is to decipher this circuitry and understand how each type and site of histone modification signals the appropriate response. The powerful *in vivo* system used by Ma *et al.* [19] will be an important tool in addressing many crucial questions about the regulatory role of histone methylation, the relationship between histone methylation and acetylation, and how these individual modifications lead to gene activation.

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