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# *In Silico* Post-Translational Modification of Histone H1: Regulation of Transcription

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**Abstract.-** Histones are a family of proteins that organize eukaryotic DNA into a compact chromatin. The linker histone, H1, is located in the interior of the folded structure, where the DNA enters and leaves the nucleosome and it possesses a well-defined three-dimensional structure. Protein methylation and acetylation are important reversible post-translational modifications of proteins, which govern cellular dynamics and plasticity and often induce conformational changes that allow the proteins to specifically interact with other proteins. Interplay of methylation and acetylation in proteins at the same Lys or Arg residues may result in regulating the gene expression and transcription. Experimental identification of the methylation and acetylation sites is often restricted due to availability of material particularly in case of transitory proteins. Computational assistance facilitates the identification and prediction of potential modification sites in proteins, in histone H1 particularly, the methylation sites will ease and provide insight for further experimental studies. The present study focuses on methylation and acetylation of histone H1, on Lys and Arg residues and describes the sites for methylation.

**Key words:** Linker Histone H1, acetylation, methylation, gene expression, post-translational modifications, PTMs.

## INTRODUCTION

Eukaryotic DNA is organized in a complex structure of chromatin. The primary function of chromatin is compaction of DNA in a manner that DNA is potentially accessible to factor-mediated regulatory responses. The assembly of chromatin into a higher-order structure plays a critical role in the control of gene transcription (Brian *et al*., 2003). Histones are a family of basic proteins that organize eukaryotic DNA into a compact chromatin. There are five major classes of histone, the core histones H2A, H2B, H3, and H4, and the linker histones, H1 (Raouf *et al.,* 2003). Two of each of the four core histones constitutes an octamer unit of the nucleosome particle. The linker histone has been shown to be located in the interior of the folded structure (Diego *et al*., 2003) where the DNA enters and leaves the nucleosome (Wolff *et al.*, 1997). Linker histones possess a well-defined three-dimensional structure, a short amino-terminal random-coiled basic portion of the molecule that is followed by a structured globular domain and a long carboxyl-terminal unstructured basic tail (Zlatanova *et al*., 1996). The globular domain is situated at or near the entry-exit of the DNA into the particle, although there are at least three models for its exact location (Widom, 1998).

Eight subtypes of linker histones are found (or described) in mice and humans, H1a, H1e, H1o, H1t (Lennox *et al*., 1983), (Zlatanova *et al*., 1994). The genomic organizations of histone H1 genes are conserved (Tanaka *et al*., 2001). H1 histones bind to DNA in the nucleosome and to the linker DNA between nucleosomes, thereby facilitating the compaction of nucleosomes into a 30-nm chromatin fiber and higher-order chromatin structures, the solenoid structure (Usachenko *et al*., 1996). These interactions between histones and DNA modulate gene activity, and both core and H1 histones have profound effects on transcription (Kiyoe *et al*., 1997).

The linker histone H1 act as general repressor of transcription by stabilizing the higher order

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structure of the chromatin (Brown *et al.*, 1996). Transcriptionally active chromatin contains less amount of H1, and removal or remodeling of H1 may be a requirement for recruitment of histone acetyl transferases (HATs) for gene activation (Bresnick *et al.*, 1992). Post-translational modification (PTM) of H1 affects PTMs of core histones (Frédéric *et al.*, 2002). Acetylation of H1 promotes acetylation of core histone H3 (Ridsdale *et al.*, 1990), whereas methylation of H1 prevents or inhibits phosphorylation and acetylation of core histones (Usachenko *et al.*, 1996).

H1 shares sequence identity with H5 (Lennox *et al.*, 1983), and it is involved in regulating gene repression (Roche *et al.*, 1985). In cancerous cells an overproduction of H1 resulted in inhibition of the expression of a number of genes (Lennox and Cohen, 1984) including c-*fos*, c-*myc*, cyclin D2 and *cdc2* (Chen *et al.*, 1987). These effects are manifested at the level of chromatin structure, as these are immediate early serum response genes and transitions in chromatin structure are associated with expression of these genes (Feng *et al.*, 1990).

Protein methylation occurs on the nitrogen atoms of either the backbone or side-chain (N-methylation) of amino acids, such as Lys, Arg, His, Ala and Asn etc (Bedford *et al.*, 2005). Considerable work utilizing this aspect has been done in linking histone modifications with chromatin dynamics in transcription (Thorsten *et al.*, 2002). Histone methylation occurs both on Lys and Arg residues (Emmy *et al.*, 1998). Several studies have suggested role of histone methylation in signal transduction and RNA metabolism however, the precise function of histone methylation remains is largely unclear. Compared to acetylation, Lys methylation of histones is known to be a stable modification (Kiyoe *et al.*, 1997). Methylation of linker histone at Lys results in chromatin condensation and contributes to repressed state. In methylation the nitrogen atom of Lys is bonded to a tetrehydral methyl carbon and positive charge is maintained. In acetylation nitrogen atom of Lys residues, form an amide bond with the carbon of acetyl group and neutralize the positive charge. This modification induces transcription (Paul *et al.*, 2002).

Acetylation of histones is linked to activation of transcription, while deacetylation is concomitant with repression of transcription (Ura *et al.*, 1997). Acetylation occurs on Lys residues within the basic amino N-terminal tail domains of the core histones. These lie towards the outside of the nucleosome (Halmer *et al.*, 1996). Histone hyperacetylation directs an allosteric change in nucleosome conformation destabilizes higher-order structure and renders nucleosomal DNA more accessible to transcription factors (Akash *et al.*, 1995). These structural transitions are a consequence of the reduction in the capacity of the acetylated N-terminal tails to stabilize the path of DNA in the nucleosome through charge neutralization. Thus acetylation of the histones destabilizes chromatin structure, alleviating repressive histone–DNA interactions and facilitating the transcription process (Alan *et al.*, 2000). The relationship between H1-mediated chromatin modulation and reversible core histone acetylation has received little attention. Acetylation appears to alter the interaction of linker histones with chromatin and may compromise the ability of H1 to promote the formation of condensed structures (Chang *et al.*, 2000). Deacetylation is required for chromatin maturation; histone acetylation apparently affects chromatin organization at a level distinct from that of core particle or linker by altering higher order structure. The focus of this study is regulation of transcription by linker histone H1 methylation and acetylation of Arg and Lys residues.

## MATERIALS AND METHODS

The sequence data used to predict potential methylation and acetylation sites of histone H1 of *Mus musculus* was retrieved from the SWISS-PROT sequence database (Boeckmann *et al.*, 2003).

The entry name was H1 MOUSE with the primary accession number P10922. BLAST search was made using NCBI database which finds regions of local similarity among the sequences of proteins or nucleotides, and can be used to elucidate evolutionary relationships (Altschul *et al.*, 1997). The search was performed on known species of different mammals. Five H1 sequences with highest bit score values were selected. The mammals selected were *Homo sapiens* (AAH29046.1), *Mus* *musculus* (P10922), *Bos taurus* (Q0IIJ2), *Rattus norvegicus* (NP_036710.1), and *Pongo pygmaeus* (Q5NVN9).

All the five sequences were multiple aligned using ClustalW (Thompson *et al*., 1994). ClustalW is a general purpose multiple sequence alignment program for DNA or proteins (Thompson *et al*., 1994).

For the prediction of methylation sites in histone H1 of *Mus musculus* MeMo 2.0 server was used (Dariusz *et al*., 2005). MeMo 2.0 (Huang *et al*., 2006) predicts methylation on the $NH_3$- functional group of Lys and Arg residues by using SVM.

## RESULTS AND DISCUSSION

The *N*-terminal domains of histone polypeptides are subject to multiple PTMs. These modifications influence the transitions between the open and compacted chromatin states. Lys methylation plays an important role in epigenetic inheritance of chromatin states (Jenuwein and Allis, 2001). Lys residues can be mono-, di- or tri-methylated by histone Lys methyltransferases (Lee *et al.*, 2004).

**Table 1: Predicted methylation sites in H1 of *Mus musculus.* K indicates Lys.**

| Residue | Flanking sequences |
|---|---|
| K20 | KRAKASKKSTDHPKY |
| K26 | KRAKASKKSTDHPKY |
| K107 | KKSTDHPKYSDMIVA |
| K108 | PKRSVAFKKTKKEVK |
| K126 | KRSVAFKKTKKEVKK |
| K131 | PKKAAKPKKAASKAP |
| K138 | KPKKAASKAPSKKPK |
| K143 | KAPSKKPKATPVKKA |
| K165 | KPKATPVKKAKKKPA |
| K173 | PVKASKPKKAKTVKP |
| K174 | VKASKPKKAKTVKPK |

The potential for methylation, at Lys and Arg residues, was predicted in H1 in *Mus musculus*. A total of 11 residues were predicted to be methylated as shown in Table I. These included only Lys residues and none of Arg residues were predicted to be methylated. In histone H1, there are 52 Lys, which is 26.94% of total amino acids, and 7 arginine residues, which is 3.62% of the total amino acids. Out of these total of 52 Lys residues, 11 (21.5%) had the potential to be methylated. Two predicted Lys residues were in the *N*-terminal region and the remaining predicted Lys residues were in the globular and *C*-terminal domain of the Histone H1. Analysis of the *C*-terminal for the conserved sequences between individual histone H1 variants, the C-terminal is diverged but the sequences of the individual *C*-termini are well conserved between all the selected species. The centrally located globular domain is the most conserved region among histone H1 family members. The *C*-terminal domain of histone H1 is responsible for high-affinity binding of histone H1 to chromatin and that high-affinity binding can be directly modulated by acetylation and methylation at the predicted residues. The globular domain is situated near the entry-exit of the DNA into the nucleosome and binding of globular domain at this position allows the *C*-terminal tail to interact with both strands of linker DNA.

Histone functions are regulated by PTMs. The linker histone H1 becomes extensively phosphorylated during entry into mitosis, and is important for proper chromatin condensation (Hsu *et al.,* 2000). Furthermore, interplay between phosphorylation and methylation on histone H1.4 occurs (Zlatanova *et al.,* 1996). This "phospho-switch" model act as a recognition site for HP1, a non-histone protein required for proper functional and structural organization of heterochromatin, where phosphorylation inhibits binding of HP1 to H1 and methylation exhibit the opposite effect (Harold *et al.,* 2001).

During the assembly of nucleosomes, histone acetylation regulates the binding of histone H1 and chromatin condensation. Displacement of histone H1 is required prior to acetylation of target genes and activation of transcription, because histone H1 inhibits histone H3 acetylation by hindering the access of histone acetyltransferases to the histone H3 tail (Julio *et al.,* 2000). The displacement of histone H1 would prevent its hyperphosphorylation and allow for chromatin decondensation, histone acetylation, and eventually, transcription activation (Kiyoe *et al.*, 1997).

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Mus musculus           TENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSI   49
Rattus norvegicus     MTENSTSTPAAKPKRAKAAKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSI   50
Bos taurus            MTENSTSTPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSI   50
Pongo pygmaeus        MTENSTSAPAAKPKRAKASKKSTDHPKYSDMVVAAIQAEKNRAGSSRQSI   50
Homo sapiens          MTENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSI   50
                      ******:**********:************:******************

Mus musculus          QKYIKSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDE   99
Rattus norvegicus     QKYIKSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKGDE   100
Bos taurus            QKYIKSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKSDE   100
Pongo pygmaeus        QKYIKSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKSDE   100
Homo sapiens          QKYIKSHYKVGENAHSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKSDE   100
                      **************.********************************.**

Mus musculus          PKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSKKPKATPVKKAKKKP   149
Rattus norvegicus     PKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSKKPKATPVKKAKKKP   150
Bos taurus            PKRSVAFKKTKKEVKKVATPKKAAKPKKAASKAPSKKPKATPVKKAKKKP   150
Pongo pygmaeus        PKKSVAFKKTKKEIKKVATPKKASKPKKAASKAPTKKPKATPVKKAKKKL   150
Homo sapiens          PKKSVAFKKTKKEIKKVATPKKASKPKKAASKAPTKKPKATPVKKAKKKL   150
                      **:* ********:*********:**********:**************

Mus musculus          AATPKKAKKPKVVKVKPVKASKPKKAKTVKPKAKSSAKRASKKK 193
Rattus norvegicus     AATPKKAKKPKIVKVKPVKASKPKKAKPVKPKAKSSAKRASKKK 194
Bos taurus            AATPKKTKKPKTVKAKPVKASKPKKTKPVKPKAKSSAKRTGKKK 194
Pongo pygmaeus        AATPKKAKKPKTVKAKPVKASKPKKAKPVKPKAKSSAKRAGKKK 194
Homo sapiens          AATPKKAKKPKTVKAKPVKASKPKKAKPVKPKAKSSAKRAGKKK 194
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Fig. 1. Sequence alignments of Histone H1 in mammals. The predicted residues for methylation and acetylation are highlighted in green.

Histone H1 condenses the chromatin by inducing the folding of the polynucleosomal fiber into ~30 nm (in diameter) structure (Ramakrishnan *et al.*, 1997). The polynucleosomal fiber lacking linker histones is only able to fold to a certain extent (Monika *et al.*, 2003), additional folding into the 30 nm fiber, under physiological conditions, can only occur on binding of histone H1 to the linker DNA. The globular and *C*-terminal domains of linker histone H1 are able to fold the chromatin fiber, but the globular domain of histone H1 alone is unable to condense the chromatin fiber. This shows that the *C*-terminal domain is important in folding of the chromatin structure. Evolutionary studies revealed that Lys-rich proteins like linker histone of Eubacteria were involved in DNA condensation (Harold *et al.*, 2001). This suggests that the *C*-terminal domain of linker histone H1 are involved in condensation of DNA.

The linker histone H1 is modulated by different PTMs, where the *C*-terminal end is most crucial for chromatin condensation and hence gene repression. Our predicted methylation sites are mostly found in the *C*-terminal of H1 in *Mus musculus* (Table I). When H1 is methylated it binds to the linker DNA and the core histones, and thereby exhibit gene repression. During gene activation H1 is demethylated and acetylated, and H1 is replaced in the chromatin by high mobility group nucleosomal proteins 1 and 2 (Frédéric *et al.*, 2002). H1 variants differ in their affinity for DNA and chromatin, and they vary in their ability to stabilize chromatin folding and repress gene activity. Different H1 variants are, in some cases, segregated in blocks in the genome, providing a molecular basis for differential folding stability and repression of entire chromosome domains. The non-acetylated and methylated histone tails carry a high positive net charge and interact with the negatively charged DNA. This interaction makes the DNA inaccessible to the transcription machinery. Acetylation or phosphorylation of the histone tails reduces the positive net charge, decreasing the interaction with the DNA. This exposes the promoter to the transcription machinery. The modified histone tails also create an interface for the recruitment of transcription factors. In conclusion interplay between methylation and acetylation of Lys residues in the *C*-terminal end of linker histone H1 may occur, and this interplay is important in the functional regulation of the protein.

We have predicted the potential methylation sites in H1, where the majority of sites were located in the C-terminal region. The C-terminal region plays a crucial role in condensation of the chromatin, and we propose that, when methylation occurs in the C-terminal of H1, the protein binds to the chromatin and the gene is repressed.

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