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Cell, Vol. 109, 801–806, June 28, 2002, Copyright ©2002 by Cell Press

Histone Methylation: Dynamic or Static?

Review

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Methylation of histones mediates transcriptional silencing at heterochromatin sites and affects regulated transcription at euchromatic loci. So is the methyl group a permanent mark on histones, or can it be removed by an active process necessary for regulated gene expression?

Gene activation is a dynamic process. It needs to be, so that the cell can respond rapidly to physiological signals. However, the cell also needs to compact its DNA in order to fit it into the nucleus. Here, then, is the problem: part of the compaction process involves wrapping approximately 1.75 turns of DNA around the histone octamer (comprising the histones H2A, H2B, H3, and H4), forming a nucleosome, which is repressive to transcription. As a result, the signals that mediate rapid transcriptional responses have to overcome nucleosomal repression before they can influence the transcriptional machinery (Wolffe, 1998).

A solution to this problem comes in the form of covalent modifications that are deposited on the N-terminal "tails" of the histones by various signaling pathways (Strahl and Allis, 2000). Such modifications (acetylation, methylation, phosphorylation, ubiquitination) can modulate the effect of the tails on chromatin compaction. Crystallographic analysis of the nucleosome has shown that the histone tails are external to the core structure and are therefore accessible for protein-protein interactions (Luger et al., 1997). They can form contacts with adjacent nucleosomes and they can act as a platform for nonhistone proteins such as HP1 or NuRD (Bannister et al., 2001; Lachner et al., 2001; Zegerman et al., 2002), which may influence chromatin compaction. Covalent modifications regulate the interaction of these proteins with the tails and in this way can overcome the negative effect of nucleosomes on transcription.

Two of the covalent modifications affecting the tails, acetylation and phosphorylation, have been shown to be reversible. Consequently, if the presence of a modification influences transcription in a particular way, its removal may have the opposing effect. In this way the cell could effectively respond to changes in environmental cues. This rapid response regulation is not thought to be available for the process of methylation. Methylation has been suggested to be biochemically stable and irreversible not at least because demethylases have not been identified yet. In other words, methylation could be a dead-end modification. Once you have it, there is no way back. In this review we survey the evidence for and against the reversible nature of methylation and consider ways in which this modification may be displaced from the histones if methylation were to be dynamically controlled.

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Methylation of Histones

The major methylation sites within histone tails are the basic amino acid side chains of lysine and arginine residues (Kouzarides, 2002; Zhang and Reinberg, 2001). The positions of many of these methylated residues within the histone N-terminal tails have now been mapped (Figure 1A), and the histone methyltransferases (HMTs) performing these modifications have recently become the focus of intense research. All known HMTs utilize S-adenosyl-methionine as the methyl group donor.

In vivo, methylated lysines can be found either in a mono-, di-, or trimethylated state (Figure 1B), whereas arginines can be either mono- or dimethylated (which can be asymmetric or symmetric) (Figure 1C). Several studies on bulk histones have indicated that mammals possess different ratios of methylated species of lysine and arginine, depending on the cell type or tissue source (Borun et al., 1972; Byvoet, 1972; Byvoet et al., 1972). The precise methylation status of a given histone tail, on a given gene, can change during the process of transcriptional activation (Kouzarides, 2002; Zhang and Reinberg, 2001). If we consider from Figure 1A that there are at least nine different positions on histones which can be methylated (a conservative estimate), then we can expect a combinational complexity of 262,144. Each of these different combinational states may dictate a distinct level of transcriptional regulation, a prediction suggested from the fact that gene activity increases when di-me K4 goes to tri-me K4 (H. Santos-Rosa, R.S., A.J.B., J. Sherriff, B. Bernstein, S.L. Schreiber, J. Mellor, and T.K., unpublished). Of course, not all these states are likely to be available alternatives to a given gene, but the exercise provides an indication as to the possible scale and complexity of histone methylation.

Is Methylation a Permanent Mark?

The dogma at present is that methylation is an irreversible process. This notion comes from early studies looking at the turnover of methyl groups in bulk histones. A number of studies conclude that histone methylation is not reversible since the half-life of histones and methyl-lysine residues within them are the same (Byvoet, et al., 1972; Duerre and Lee, 1974). If, as these studies imply, a methyl group cannot be removed from a histone via an active process, it will remain on a promoter until natural histone turnover or DNA replication replaces the modified histone with an unmodified one. Such a "permanent" methyl mark is compatible with certain forms of transcriptional silencing, such as those found at centromeric heterochromatin and at DNA-methylated promoters (Kouzarides, 2002). For these types of genes,

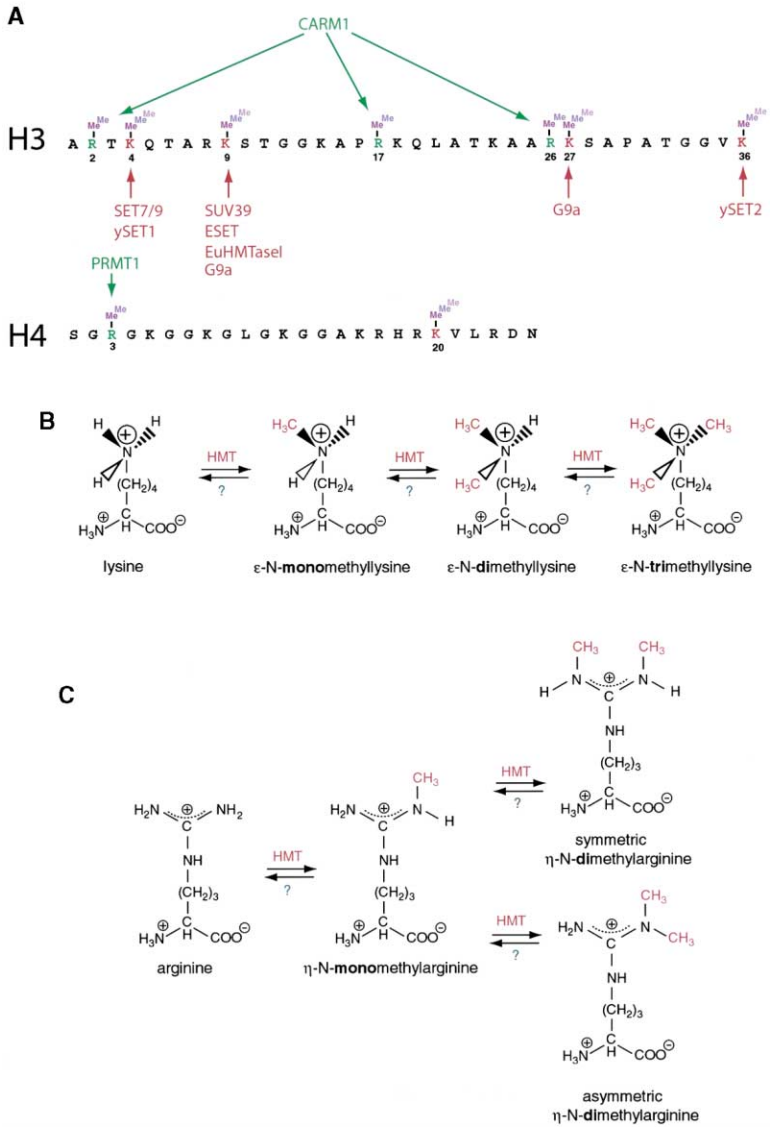

Figure 1. Sites and Structures of Methylated Residues in Histone Tails

(A) Known sites of methylation in the N-terminal tails of histones H3 and H4. Lysines found to be methylated are shown in red and arginines are shown in green. Lysines have the potential to be mono-, di-, or trimethylated. Known histone methyltransferases and their preferred methylation sites are also indicated.

(B) Chemical structures of lysine and its methylated derivatives. The action of histone methyltransferases (HMTs) is indicated. The potential reversibilty of the reaction by demethylases is indicated with question marks.

(C) Chemical structures of arginine and its methylated derivatives. The action of histone methyltransferases (HMTs) is indicated. The potential demethylation reactions are indicated with question marks. The two forms in which dimethylarginine can be found are symmetric or asymetric.

the silencing is considered to be a long-term state, and methylation of K9 H3 has been implicated in this process. A stable methyl group also makes sense as part of an epigenetic mark for the inheritance of the silenced state following replication (Zhang and Reinberg, 2001). Furthermore, it is possible that a methylated histone tail may render the histone more or less susceptible to an additional, reversable modification such as acetylation.

There is, however, some evidence that active turnover of methyl groups does take place at a low but detectable level (Borun et al., 1972; Annunziato et al., 1995). The existence of a process that reverses histone methylation is certainly necessary when considering the role of methylation more in regulated gene expression. The appearance of a methyl group on either lysines or arginines has been correlated with genes being activated or repressed. So how can rapid reversal of gene expression take place, if the methyl group has a half-life as long as that of histones? In other words, if methylation plays a direct role in gene expression, then alteration of genes' activity status should be concomitant with a change in its methylation status.

There are several documented cases in which active reversal of methylation may be deemed to be necessary, if methylation is directly implicated in a gene's expression level. One such case is the cyclin E promoter, whose activity is repressed by K9 H3 methylation in the G1 phase of the cell cycle (Nielsen et al., 2001). This promoter is activated at the G1/S phase transition, so, in principle, K9 methylation should be reversed to allow cyclin E to be expressed. Another example comes from yeast, where active promoters are trimethylated at K4 H3, but when they become repressed they are dimethylated (H. Santos-Rosa, R.S., A.J.B., J. Sherriff, B. Bernstein, S.L. Schreiber, J. Mellor, and T.K., unpublished). How does the methyl state change from tri-me to di-me? A third example comes from nuclear hormone receptor-regulated genes such as pS2 whose promoter is rapidly activated by estrogen, and methylation at R17 H3 occurs concomitantly (Bauer et al., 2002; Strahl et al., 2001).

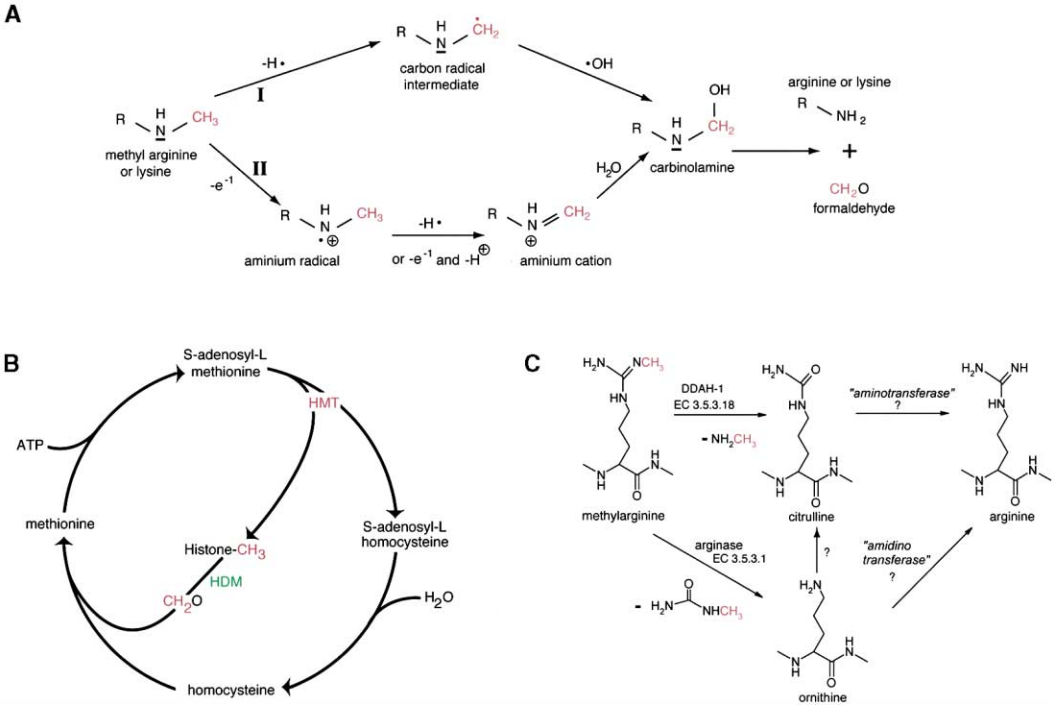

Figure 2. Potential Chemical Pathways for Histone Demethylation

(A) Two potential reaction pathways (I and II) for the enzymatic demethylation of methyl lysine or arginine. R stands for the rest of the amino acid. The methyl group is shown in red.

(B) A proposed scheme for the recycling of the formaldehyde generated in the demethylation reaction into S-adenosyl-L methionine. Histone demethylases (HDMs) act to demethylate histones, thereby generating formaldehyde, which is transferred to a homocysteine to generate methionine, which is then cycled back to S-adenosyl-L methionine.

(C) Enzymatic demethylation pathways specific to methyl arginine. The demethylation products citrulline or ornithine could be cycled back to arginine.

However, these promoters can also be rapidly turned off following the addition of antagonists such as heregulin. What happens to the methyl group on R17 when the gene is repressed? If methylation of R17 H3 mediates the active state, then we would expect it to disappear when the gene becomes turned off again.

Rapid reversibility of methylation has not been documented in the above cases, so the jury is still out on the "permanency" for these methyl marks. If it is found, then this would be evidence for an active reversal of methylation. If it is not, then the chances are that methylation is merely a tagging process, marking genes, which are either on or off. The main players in transcriptional control may be the proteins that they recruit. Regulating their binding to the methyl group (say, by posttranslational modification) could be the determinant of whether a gene is activated or repressed.

Potential Mechanisms of Active Methyl Turnover

It is not unreasonable to consider the possibility that methylation may be actively reversed in a controlled, signal-dependent way given the arguments above. If methylation is dynamic, how could its reversibility be regulated?

Demethylation

The most straightforward way to rapidly reverse methylation is by the enzymatic action of a demethylase. A hunt for such an enzyme was carried out over thirty-five years ago. In 1964, Paik and coworkers published the purification of an enzyme (N6-methyl-lysine oxidase, EC number 1.5.3.4) from rat kidney capable of demethylating free mono- and di-N-methyllysine (Kim et al., 1964). The same group reported a few years later the detection of an enzyme capable of demethylating histones (Paik and Kim, 1973). Soon after, they partially purified this enzymatic activity but could not ultimately relate the activity to a specific protein (Paik and Kim, 1974). More recently, Chinenov has shown that the yeast histone acetyltransferase and transcriptional elongator Elp3 has sequence similarity to an enzyme superfamily that uses S-adenosyl-L-methionine (SAM) in radical reactions (Chinenov, 2002). Based on this similarity, it was suggested that Elp3 might be a histone demethylase. Other enzymes with demethylase activity include the N-methyltryptophan and sarcosine demethylases (Khanna and Jorns, 2001; Wagner and Jorns, 2000). It is important to note, however, that these latter two enzymes do not demethylate amino acid side chains within proteins, but nevertheless their characterized reaction mechanisms may be useful in the search for histone demethylase activity. All these enzyme activities, and the suggested Elp3 demethylation reaction, result in the production of formaldehyde. Thus, these demethylation processes probably occur via an oxidative mechanism.

Based on these reports, two potential reaction pathways (I and II) can be considered for the removal of

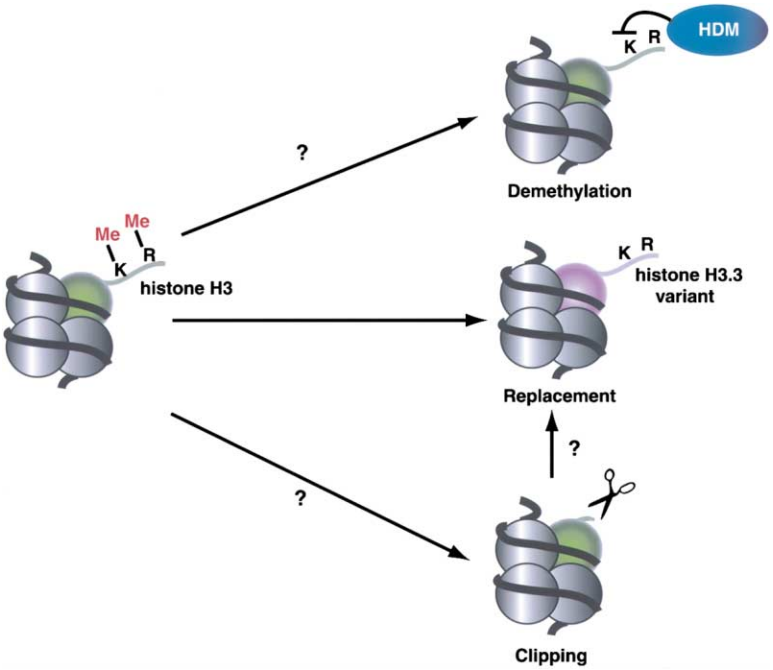

Figure 3. Schematic Representation of Various Pathways for Active Methyl Group Turnover

Enzymatic demethylation, replacement of H3 by replacement variant H3.3, and clipping of the N-terminal tail are shown.

methyl groups from both lysines and arginines (Figure 2A). Pathway I begins with a single electron oxidation step (as occurs with cytochrome P450 enzymes) (Ortiz de Montellano, 1995) leading to the formation of a carbon radical intermediate. The next step of this pathway involves the addition of an OH radical, which could be supplied by a metalloenzyme, to form a carbinolamine intermediate. The alternative pathway II is based on a two electron oxidation process via an aminium radical that is then converted to an aminium cation. This is then hydrolyzed, probably in a nonenzymatic process, to the common intermediate carbinolamine, which readily breaks down to formaldehyde and lysine or arginine. Both of these schemes could use a FAD cofactor as the electron acceptor. It is interesting to note that pathway II is not open to trimethylated lysine, but can be used for mono- and dimethylated. If both of these demethylation pathways are operational in vivo, then this may suggest the existence of demethylases that can discriminate between the di- and trimethyl states.

If demethylation leads to the formation of formaldehyde, then this reactive product would react instantly in vivo with any amine. However, a cycle can be envisaged whereby the formaldehyde can be cycled back to SAM (Figure 2B). In this way the formaldehyde by-product of demethylation could be used as the methyl donor for histone methyltransferases (HMTs) (Figure 2B), thus forming a closed reaction loop (Tyihák et al., 1998).

For methyl arginine there are alternative hydrolytic/amidation pathways open for demethylation that are not open for lysines (Figure 2C). An enzyme exists (L-Nw, Nw-dimethylarginine dimethyl-aminohydrolase-1 [DDAH-1]) that catalyzes the hydrolysis of monomethyl and dimethyl (asymmetric) arginine to citrulline (Ogawa et al., 1989). Since citrulline exists in histones (Hagiwara et al., 2002), it is tempting to speculate that an enzyme similar to this may function to demethylate histone arginines. Citrulline may be the endpoint of arginine demethylation until the histone is ultimately replaced (see below), or alternatively, an enzyme may convert citrulline back to arginine (Figure 2C), as happens in certain biosynthetic pathways.

A second arginine-specific demethylation may occur via its conversion to ornithine (Figure 2C). Precedent for this type of reaction is found in the urea cycle and is performed by arginine amidinase. However, unlike DDAH-1, it is unclear whether this class of enzyme can deamidinate methylated arginines, although of course a distinct amidinase-family member may exist that possess this ability. As with histone citrulline, the ornithine product could be the demethylation endpoint. It may also be possible to reconvert the ornithine back to arginine either via the addition of an amidine fragment or via its conversion to citrulline and then to arginine as described above.

Histone Replacement

An alternative mechanism for a response-mediated removal of a methyl group is the replacement of the modified histone with an unmodified version. The standard core histone genes are coordinately upregulated at the onset of S phase, consistent with histone deposition during DNA replication. In general, the more likely candidates for a replacement-mediated methyl turnover are the histone variants of which there are many genes in mammalian cells. Some of these have been described as replacement variants, which unlike histones are constantly transcribed throughout the cell cycle (Waterbourg, 1993; Wunsch and Lough, 1990; Yu and Gorovsky, 1997). Since these variant histones are almost identical to their standard counterparts except for very few amino acid substitutions (only four in the histone H3 variant H3.3), they make perfect candidates for substitution partners for standard histones (Figure 3). Indeed, there is evidence that histone variants (e.g., histone

H3.3) can be deposited in chromatin in a replication-independent manner, i.e., through the cell cycle and not just at S phase (Thatcher et al., 1994). In particular, the histone H3 variant H3.3 is deposited in chromatin in brain cells that have exited the cell cycle (Pina and Suau, 1987). In *Tetrahymena*, histone H3.3 is only present in the macronucleus where transcription occurs, which led Gorovsky and colleagues to suggest a replacement function for H3.3 during transcription (Yu and Gorovsky, 1997).

Clipping

Evidence from *Tetrahymena* has pointed to the existence of a clipped version of histone H3 specifically within the micronucleus, where there is no transcription. This proteolytically cleaved histone H3 lacks the first six N-terminal residues (Allis et al., 1980), thus removing R2 and K4, both of which are potential sites for methylation and marks for activation. This may therefore represent a mechanism by which methylation can be removed in an active process prior to the replacement of histones during DNA replication. This mechanism does not allow for a dynamic regulation of methylation, but may just represent a way of eliminating the consequences of methylation at any point in the cell cycle. On an even more speculative note, this cleavage may well regulate methylation elsewhere in the histone if removal of methylated R2 or K4 has repercussions on downstream methylation events.

Alternatively, the clipped H3 may not be an end product in itself, but may represent an intermediate state for the replacement of H3 by a histone variant. In this scenario a histone to be substituted by a variant is recognized by the replacement machinery by virtue of its clipped N terminus (Figure 3).

Concluding Remarks

In this review we have presented the available evidence, which suggests that the methyl group is a stable mark on histones. The experimental evidence comes from examining bulk histones, a process that may not necessarily detect small changes in methylation. The question of permanency may need to be revisited using more sensitive techniques involving the analysis of individual genes. The need for further exploration of the methyl-turnover issue comes from tantalizing, but not yet firm, evidence that methylation is involved in dynamic changes in gene expression.

Assuming that active turnover is a possibility, we have put forward several scenarios of how this turnover may take place based on evidence from the literature. The demethylation reactions are chemically plausible and based on experimental investigations, but may only be a subset of the possibilities open to this process. We hope that highlighting these reactions will help catalyze further investigation into the potential existence of these enzymes. The conserved domain found within the yeast Elp3 protein (Chinenov, 2002) has recently been postulated to represent a demethylase domain. Clearly this has to be investigated further, and hopefully more widespread searches will also be used to identify such enzymes. The replacement and clipping scenarios are also plausible in their own right, rather than merely alternatives to a demethylation reaction. The cell may have evolved several ways to deal with a "permanent" methyl mark, and there may be many other ways to displace this group from the histones in an active fashion. Our hunch is that active turnover is likely to take place in some form. Although this statement is based on speculation, as Goethe wrote, "speculative ideas are like chess pieces moved forward, they may be beaten but they may start a winning game."

Acknowledgments

We thank Alan Davidson and Shankar Balasubramanian for their help with the chemical reactions involving demethylation.

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