

DNA methylation: a new twist in the tail

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DNA methylation is the epigenetic mark with the longest history and that we probably understand best, yet we still have no adequate account for why specific DNA sequences are selected to become methylated. Gene-specific DNA methylation is fundamental to processes such as developmental silencing of genes, classical epigenetic phenomena such as genomic imprinting, and occurs pathologically in the silencing of tumor suppressor genes in cancer. Fully understanding the mechanisms of methylation is thus of huge importance. In mammals, the acquisition of DNA methylation is determined by one of two *de novo* DNA methyltransferase enzymes, Dnmt3a and Dnmt3b. These activities are assisted by the related, but catalytically inactive protein Dnmt3L. Dnmt3a and Dnmt3b have similar structures, comprising a PWWP domain, PHD-like or ADD domain and a carboxy-terminal catalytic domain [1]. The PWWP domains are required for binding of Dnmt3a and Dnmt3b to chromatin *in vivo*, and the PHD domain is thought to be dispensable for this [2]. Dnmt3L, which is a truncated protein lacking the PWWP and a functional catalytic domain, forms a heterotetramer with Dnmt3a or Dnmt3b [3] and is described as stimulating the activity of its *de novo*

partners, or guiding the recognition of DNA targets with a particular periodicity of CpG sites [3], or attenuating the inherent sequence specificities of Dnmt3a or Dnmt3b so that they methylate targets more homogeneously [4].

It has long been suspected that there would be cross-talk between DNA methylation and chromatin state, and a key observation came four years ago when it was shown that the PHD domain of Dnmt3L interacts with the amino-terminal tail of histone H3, but that specific methylation of the lysine at residue 4 (H3K4) inhibits this interaction [5]. This made a good deal of sense, as H3K4 methylation is a marker for active genes and there is an inverse correlation between the presence of trimethylated H3K4 and DNA methylation at promoters. Moreover, the H3K4 demethylase KDM1B is required for establishment of DNA methylation at the control regions of a subset of imprinted genes [6]. Subsequently, similar properties of the PHD domains of Dnmt3a and Dnmt3b have been described [7]; in addition, the PWWP domain of Dnmt3a specifically recognizes trimethylated H3K36 [8]. Together, these observations suggest that an appropriate combination of histone modifications serves as a docking platform for the *de novo* methylation complexes. The study by Li and colleagues [9] published recently in *Cell Research* adds an interesting new twist to the mechanism by which histone modifications might contribute to *de*

*nov*o DNA methylation.

The authors found that amino-terminal peptides of H3 not only bound the PHD domain of Dnmt3a, but also actually stimulated its activity *in vitro*. This effect was sensitive to the modification state of lysine 4, as stimulation was progressively reduced with increasing methylation and abolished by trimethylation. To demonstrate the *in vivo* significance of this observation, Dnmt3a carrying point mutations in residues essential for H3 tail binding, but not affecting basal enzyme activity *in vitro*, were stably transfected into mouse ES cells lacking endogenous Dnmt3a and Dnmt3b and were shown to be incapable of methylating the *Oct4* promoter upon differentiation, although binding to *Oct4* was apparently not affected. These initial results were interpreted to suggest that binding of the H3 tail causes allosteric activation of Dnmt3a. To investigate this possibility further, the authors mutated residues predicted to be involved in the interfaces between the PHD and catalytic domains of Dnmt3a. Two residues were identified at which substitutions strongly impaired the ability of H3 peptides to stimulate Dnmt3a activity *in vitro*, again without affecting basal activity. These mutations were then tested in the *in vivo* setting. Importantly, neither mutation altered the ability of Dnmt3a to complex with Dnmt3L, or its binding to the *Oct4* promoter or major satellite DNA but, again, the ability of both mutants to

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methylate these target sequences was blocked, strengthening the conclusion that the interaction of H3 tails with the PHD domain of Dnmt3a is required for allosteric activation. From these results, the authors present a novel, two-step model for methylation by Dnmt3a in which the protein is recruited to chromatin via its PWWP domain, or through interactions with Dnmt3L or other chromatin-bound proteins, but is activated only by binding of the PHD domain by unmethylated H3K4.

Does this interesting new twist help us to understand how specific DNA sequences are targeted for methylation? One challenge is how to incorporate these observations into a model that includes Dnmt3a in its association with Dnmt3L. *In vivo* it appears that Dnmt3a depends largely on Dnmt3L, as revealed by genome-wide analysis of CpG island methylation in mouse oocytes [10]. The Dnmt3a:Dnmt3L heterotetramer seems to be a complex bristling with recognition sites receptive to histone tail modifications, and these might act synergistically (on single or adjacent nucleosomes?) so that at preferred genomic targets all sites are engaged with the *de novo* complex with ensuing stimulation of Dnmt3a. Is H3 tail binding to the PHD domain of Dnmt3L, which itself lacks catalytic function,

communicated within the complex to further enhance the activity of Dnmt3a? Or does the interaction of Dnmt3L with unmodified H3 tails serve primarily to help specify binding of the complex, as Dnmt3L seems to have a major influence on the subnuclear localization of Dnmt3a [11]? We might need more sophisticated assays to identify targets of Dnmt3a and their chromatin properties, and whether choice of target is modified by Dnmt3L. In any case, the study emphasizes the critical role of histone tail modification status in determining binding and activity of the *de novo* methylation complex.

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