

A Methylation Rendezvous: Reader Meets Writers

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It is well established that two marks of silent chromatin, DNA methylation and histone H3 methylation at lysine 9, engage in an epigenetic conversation. In a recent issue of *Genes & Development*, Smallwood et al. (2007) report that the mammalian HP1 adaptor “translates” methylation information from histone to DNA, helping to cement epigenetic expression states.

Methylation of cytosine bases in DNA occurs to varying degrees in a wide range of organisms, from plants to mammals. It affects many biological processes, notably genomic imprinting and X inactivation, and plays a central role in silencing gene expression in both heterochromatin and euchromatic domains. In mammals, the addition of methyl groups to cytosine is catalyzed by three active DNA methyltransferases (DNMTs).

How DNA methylation patterns are established by the DNMTs has long remained obscure. Evidence is accruing that DNA methyltransferases take at least some cues from histone modifications, as exemplified by the well-established intimate link between cytosine methylation and histone H3 methylation at lysine 9 (H3K9).

Lysine 9 of H3 can exist in a mono-, di- or trimethylated state. Mono- and dimethylation are catalyzed in euchromatic regions by the histone methyltransferase G9a, whereas H3K9 trimethylation, prevalent in pericentromeric heterochromatin domains, results primarily from the activity of the SUV39H histone methyltransferases. The H3K9-CpG methylation dialog in mammals has been evidenced by studies, among others, on Suv39h null and G9a null mouse embryonic stem cells. In the former, loss of H3K9 trimethylation reduces DNA methylation of pericentric heterochromatin. In the latter, CpG methylation is likewise reduced in several euchromatic regions (Fuks, 2005).

So, H3K9 methylation can serve as a beacon for DNA methylation, but how is the histone methylated mark relayed to the DNMTs to bring about gene silencing? It is known that, once methylated by the SUV39H enzymes,

lysine 9 of H3 functions as a docking site for an adaptor molecule, heterochromatin protein 1 (HP1), which can bind to mammalian DNMTs. This makes HP1 an attractive candidate “translator” of H3K9 methylation into DNA methylation. In a recent issue of *Genes & Development*, Smallwood and coworkers report that the HP1 protein is the “reader” that targets DNMT1 enzyme activity to euchromatic sites bearing the H3K9 dimethyl mark, thus providing a basis for the generation of CpG methylation patterns (Smallwood et al., 2007).

The study of Smallwood et al. started with in vitro interaction assays confirming and extending the previously reported direct association (Fuks et al., 2003; Lehnertz et al., 2003) of DNMTs with all three isoforms of HP1 (α , β , and γ). The authors then explored the functional consequences of these associations and found, using an immobilized template approach and DNA methyltransferase assays, that H3K9 dimethylation by G9a increases recruitment of all three HP1s, resulting in significant stimulation of DNMT1 enzymatic activity. How might HP1 increase DNMT1 activity? It does not seem to promote DNMT1 binding to DNA, but it might trigger allosteric activation of DNMT1, a known feature of the enzyme (Pradhan and Esteve, 2003). Interestingly, these experiments suggest for the first time that HP1 can bind to histone H3K9 when the latter is dimethylated by the G9a enzyme, but more thorough biochemical analyses are needed to confirm this.

These findings are also valid in vivo. The authors showed this by means of an HP1 tethering assay in a cell system well characterized for the study of

DNMT1: DNMT1-deficient HCT116 cells and their wild-type counterparts. First, they showed that HP1 requires DNMT1 for full silencing, with DNMT1 possibly helping HP1 to load onto chromatin. Second, they used an adapted methylated DNA immunoprecipitation (MeDIP) approach to show that HP1 enhances DNMT1-mediated DNA methylation of the target promoter, although it may be necessary to confirm this using alternative methods (e.g., bisulfite genomic sequencing). Lastly, chromatin immunoprecipitation (ChIP) assays revealed concomitant and DNMT1-dependent recruitment of HP1 α , HP1 β , G9a, and DNMT1 to several endogenous euchromatic promoters. It is noteworthy that HP1 γ binding to promoter, in contrast to HP1 α and HP1 β binding, correlated with gene activation. This echoes other evidence that HP1 proteins are “two-faced” in their effects on gene expression (Vakoc et al., 2006).

As DNMTs are still commonly classified as either “maintenance” (DNMT1) or “de novo” DNA methyltransferases (DNMT3), this work implying that DNMT1 acts as a “de novo” DNA methyltransferase might seem surprising. Yet it is emerging that this classification may be too simplistic, and the possibility that DNMT1 may also possess “de novo” activity (Jair et al., 2006) should be borne in mind.

As a whole, the Smallwood study supports a model where the HP1 adaptor plays an essential role in transmitting the flow of epigenetic information between two distinct methylation layers (Figure 1). In this model, the G9a enzyme mediates histone H3K9 dimethylation at euchromatic

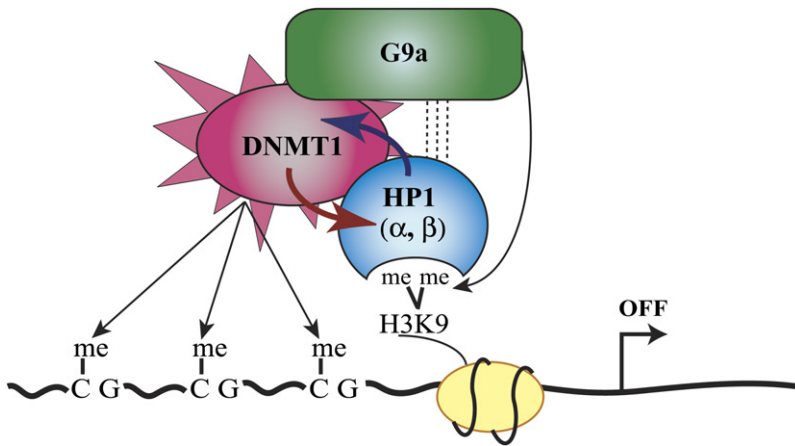


Figure 1. Hypothetical Model Depicting a Central Role for the HP1 Adaptor as a Relay between Two Distinct Methylation Layers for Reinforcing Silent Epigenetic States

Mammalian HP1 α and β are attracted to euchromatic genes in regions where lysine 9 of histone H3 (H3K9) has been methylated by the G9a histone methyltransferase. The HP1 adaptor then binds to DNMT1 and potentiates its DNA methyltransferase activity (blue arrow), thereby enhancing cytosine methylation (meCG) on nearby DNA. DNMT1 could in turn assist HP1 loading onto chromatin (red arrow). Furthermore, association of DNMT1 with G9a could allow a direct impact of DNA and H3K9 methylation states on each other. Together, these positive feedback loops could stabilize inactive chromatin, resulting in tight transcriptional repression.

genes, creating a binding platform for HP1. Bound HP1 then interacts with DNMT1, possibly potentiating its activity, and facilitates CpG methylation on nearby DNA. DNMT1 may in turn stabilize HP1 binding to chromatin, resulting in further cytosine methylation. The reported association of DNMT1 with G9a (Esteve et al., 2006) could ensure a direct impact of H3K9 dimethylation states and DNA methylation on each other. Thus, the cytosine and H3K9 methylation marks appear to generate mutual boosting and feedback loops, thereby shutting down gene expression.

Might the above model also apply to other organisms? The H3K9-CpG methylation dialog is not restricted to mammals. It was first evidenced, in fact, in the filamentous fungus *Neurospora crassa* and the plant *Arabidopsis thaliana*. In accordance with the proposed mammalian scenario, the *Neurospora* HP1 homolog is essential to DNA methylation (Freitag et al., 2004). In *Arabidopsis*, however, the DNMTs themselves seem to be recruited directly to sites of H3K9 methylation and associated modifications (Lindroth et al., 2004). In mammals, it is

worth considering another, more direct link between DNA methylation and histone methylation: histone methyltransferases like SETDB1 contain a potential methyl-CpG binding domain; it is thus imaginable that they might “back-translate” methylated DNA to methylated lysine 9 of H3. This intriguing connection seems worthy of future study.

The work of Smallwood et al. is attractive because it may shed light on how HP1 mediates gene silencing. An emerging scenario is that HP1 brings to promoters a set of enzymatic activities required for repression. Acting as an auxiliary of HP1 association with chromatin in euchromatic regions, DNMT1 might participate in this mode of silencing. It is worth stressing that H3K9 methylation is not the only means of HP1 recruitment to chromatin and that, although this is highly speculative, the DNMT1-HP1 connection might also act independently of the H3K9 mark.

In summary, the proposed model is appealing because a ménage-à-trois between the methylation “writers” G9a and DNMT1 and the HP1 “reader” might create a self-propagating epige-

netic cycle that firmly locks genes into a “repressed” state. These findings are an invitation to elucidate in detail the links and crosstalk between the H3K9 and DNA methylation layers. The emerging picture does not resemble a unidirectional signaling pathway between histones and DNA. It suggests, rather, a complex interplay between mutually influencing marks. It is clear, furthermore, that cytosine methylation and histone methylation each have some degree of mutual autonomy, varying according to the context. The picture that is forming is one of a conversation full of subtle inflections, with multiple partners and mediators. The epigenetic rendezvous depicted in Figure 1 is doubtless too simplistic, and this model is bound to become much more elaborate in the near future.

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