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Suv39h-Mediated Histone H3 Lysine 9 Methylation Directs DNA Methylation to Major Satellite Repeats at Pericentric Heterochromatin

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Summary

Background: Histone H3 lysine 9 (H3-K9) methylation and DNA methylation are characteristic hallmarks of mammalian heterochromatin. H3-K9 methylation was recently shown to be a prerequisite for DNA methylation in *Neurospora crassa* and *Arabidopsis thaliana*. Currently, it is unknown whether a similar dependence exists in mammalian organisms.

Results: Here, we demonstrate a physical and functional link between the Suv39h-HP1 histone methylation system and DNA methyltransferase 3b (Dnmt3b) in mammals. Whereas in wild-type cells Dnmt3b interacts with HP1α and is concentrated at heterochromatic foci, it fails to localize to these regions in *Suv39h* double null (dn) mouse embryonic stem (ES) cells. Consistently, the *Suv39h* dn ES cells display an altered DNA methylation profile at pericentric satellite repeats, but not at other repeat sequences. In contrast, H3-K9 trimethylation at pericentric heterochromatin is not impaired in *Dnmt1* single- or *Dnmt3a*/*Dnmt3b* double-deficient ES cells. We also show that pericentric heterochromatin is not transcriptionally inert and can give rise to transcripts spanning the major satellite repeats.

Conclusions: These data demonstrate an evolutionarily conserved pathway between histone H3-K9 methylation and DNA methylation in mammals. While the Suv39h HMTases are required to direct H3-K9 trimethylation and Dnmt3b-dependent DNA methylation at pericentric repeats, DNA methylation at centromeric repeats occurs independent of Suv39h function. Thus, our data also indicate a more complex interrelatedness between histone and DNA methylation systems in mammals. Both methylation systems are likely to be important in reinforcing the stability of heterochromatic subdomains and thereby in protecting genome integrity.

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Introduction

In a variety of multicellular organisms ranging from fungi to plants to mammals, distinct patterns of DNA methylation coregulate epigenetic gene silencing and are important for maintaining genome integrity [1]. In mammals, three catalytically active DNA methyltransferases (DNMTs) have been described [2]. Whereas Dnmt3a and Dnmt3b have been shown to be required for de novo DNA methylation, Dnmt1 appears to function primarily in propagating heritable DNA methylation patterns following DNA replication [3, 4]. Since there is little intrinsic target specificity of DNMTs beyond CpG dinucleotides [5–7], it has been proposed that DNA methylation may be directed by alterations in the chromatin structure that would reflect differences between transcriptionally active and repressed regions [1]. Indeed, DNA methylation patterns are perturbed in mutants that encode components of chromatin-remodeling complexes, such as SNF2-like factors [8–11]. In addition, studies in *Neurospora crassa* [12] and in *Arabidopsis thaliana* [13] have indicated that a repressive chromatin modification, such as histone H3 lysine 9 (H3-K9) methylation, can direct DNA methylation. Whether a similar mechanistic connection is also operative in vertebrate systems and which mammalian DNA methylation patterns may be responsive to changes in H3-K9 methylation is not known.

In mice, pericentric heterochromatin is characterized by high levels of DNA methylation and H3-K9 trimethylation ([14, 15]; A.H.F.M.P., submitted). The histone methyltransferases (HMTases) Suv39h1 andSuv39h2 are the enzymes that together are required to establish H3-K9 trimethylation at these regions ([15]; A.H.F.M.P., submitted). The heterochromatin protein 1 isoforms HP1α and HP1β have been shown to bind to methylated H3-K9 residues [16–18] and are thought to function downstream of the Suv39h HMTases to stabilize heterochromatic subdomains. In this study, we demonstrate that DNA methylation of pericentric satellite repeats by Dnmt3a and Dnmt3b is dependent on a functional Suv39h-HP1 histone methylation system.

Results

Mammalian DNMTs Interact with an Associated H3-K9 HMTase Activity

To address whether Suv39h HMTase activity would affect DNA methylation profiles, we first analyzed coexpression of all three mammalian DNMTs, the methyl-CpG binding protein MeCP2 [19], HP1α, and Suv39h2 in mouse embryonic fibroblasts (MEFs) that are either wild-type (wt) or double null (dn) for both *Suv39h1* and *Suv39h2*. Immunoblots of nuclear extracts revealed that Dnmt3b is not present at detectable levels in MEFs, but

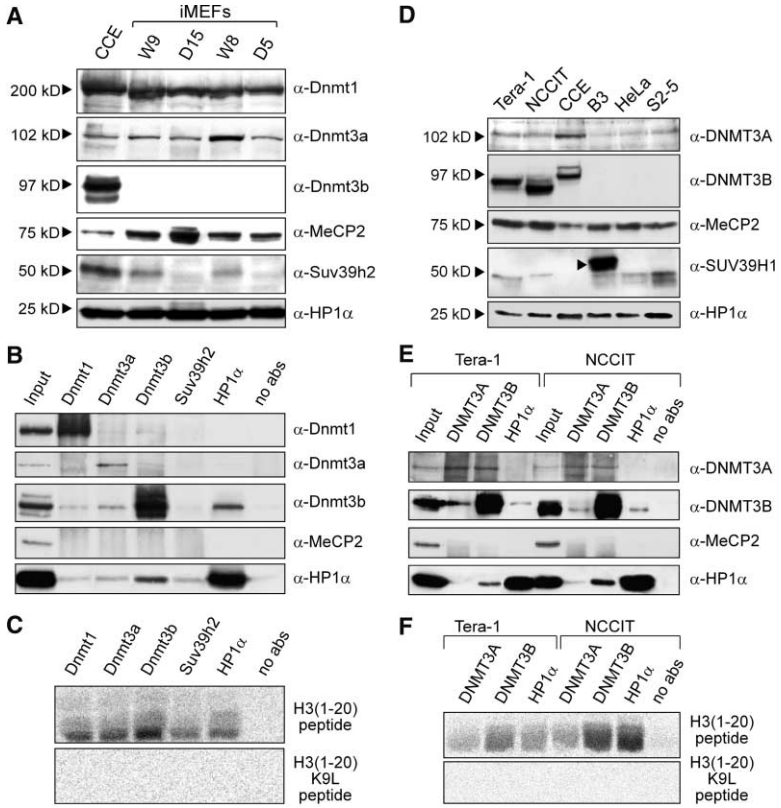

Figure 1. Biochemical Interaction between DNMTs and H3-K9 HMTase Activities in Murine CCE and Human Embryonic Carcinoma Cells

(A) Protein blots of nuclear extracts showing expression profiles for the different DNMTs, MeCP2, Suv39h2, and HP1α in CCE embryonic stem cells and in wild-type (wt; W9 and W8) and *Suv39h* double null (dn; D15 and D5) mouse embryonic fibroblasts (iMEFs).

(B) Coimmunoprecipitations from CCE nuclear extracts revealing selective enrichment between Dnmt3b and HP1α.

(C) A fluorogram showing H3-K9-specific HMTase activity associated with the various co-IPs displayed in (B).

(D) Protein blots of nuclear extracts showing expression profiles of the different DNMTs, MeCP2, SUV39H1, and HP1α in human embryonic carcinoma cells (Tera-1, NCCIT) and in HeLa cells overexpressing human SUV39H1 (B3) [39] or mouse Suv39h2 (S2-5) [40]. The Dnmt1 antibodies used in (A) and (B) do not recognize human DNMT1, and human DNMT3b in Tera-1 and NCCIT cells is present in a variety of isoforms [20].

(E) Coimmunoprecipitations from Tera-1 and NCCIT nuclear extracts revealing selective enrichment between DNMT3b and HP1α.

(F) A fluorogram showing H3-K9-specific HMTase activity associated with the various co-IPs displayed in (E).

there is significant coexpression of all analyzed proteins in the mouse embryonic stem (ES) cell line CCE (Figure 1A). We therefore used nuclear extracts from CCE cells to perform coimmunoprecipitations (co-IPs), followed by in vitro HMTase assays. Although we did not observe an interaction between Suv39h2 and any of the DNMTs, we detected a selective, yet substoichiometric, enrichment in the association between Dnmt3b and HP1α (Figure 1B). This interaction was confirmed in the reciprocal co-IP with HP1α antibodies. We also observed an interaction between Dnmt3b and HP1β, whereas HP1γ appeared not to associate with Dnmt3b (data not shown). We then used the co-IP material to examine the presence of HMTase activity that would be able to methylate the H3-K9 position, as controlled with wild-type and mutant (K9L) histone H3 peptide substrates. These HMTase assays indicated that Suv39h2, HP1α, and all three DNMT antibodies enriched for an H3-K9 HMTase activity that was most pronounced with the Dnmt3b co-IP (Figure 1C).

To examine whether the interactions between Dnmt3b and HP1α would also be present in other mammalian cells, we repeated the co-IPs with nuclear extracts from the human embryonic carcinoma cell lines Tera-1 and NCCIT, in which DNMT3A and a variety of DNMT3B isoforms are expressed [20] (Figure 1D). Similar to CCE cells, we failed to co-IP SUV39H1 and DNMT3B (data not shown); however, we again detected a specific interaction between DNMT3B and HP1α (Figure 1E) or HP1β (data not shown). Importantly, HMTase assays with the co-IP material demonstrated that DNMT3A, DNMT3B, and HP1α also associate with an H3-K9-specific HMTase activity in human cells (Figure 1F).

Pericentric Localization of Dnmt3b Depends on *Suv39h*-Mediated H3-K9 Methylation

To investigate the in vivo significance of the Dnmt3b-HP1α interaction, we generated three wild-type (wt) and three *Suv39h* dn ES cell lines by blastocyst outgrowth. Pericentric heterochromatin in mouse cells is characterized by several blocks of AT-rich repeats that can be visualized as focal nuclear stainings by fluorochrome 4',6'-diamino-2-phenylindole (DAPI). In addition, HP1α and HP1β have been shown to accumulate at these DAPI-dense regions [17]. To demonstrate heterochromatic association, we used HP1α antibodies and DAPI in double labeling immunofluorescence analyses. For example, in wt ES cells, costaining with H3-K9 trimethylation antibodies (see Figure S1 in the Supplemental Data available with this article online) (A.H.F.M.P., submitted) and HP1α antibodies resulted in significant overlap at several foci that colocalized with DAPI-rich heterochromatin (Figure 2A, top panels). Colocalization with HP1α is indicated as yellow foci, whereas costaining with DAPI is reflected by the appearance of white dots. Conversely, H3-K4 dimethylation was enriched at euchromatic regions and did not colocalize with the heterochromatic HP1α proteins in wt ES cells (Figure 2A, top panels).

Similar to fibroblasts prepared from *Suv39h* dn embryos [15], *Suv39h* dn ES cells lacked H3-K9 trimethylation at pericentric heterochromatin (Figure 2A, bottom panels). As a consequence, binding of HP1α and HP1β (data not shown) to these domains is abrogated, and no focal staining was observed. Therefore, in *Suv39h* dn ES cells, HP1α staining could not be used to evaluate heterochromatic colocalization, which instead was de-

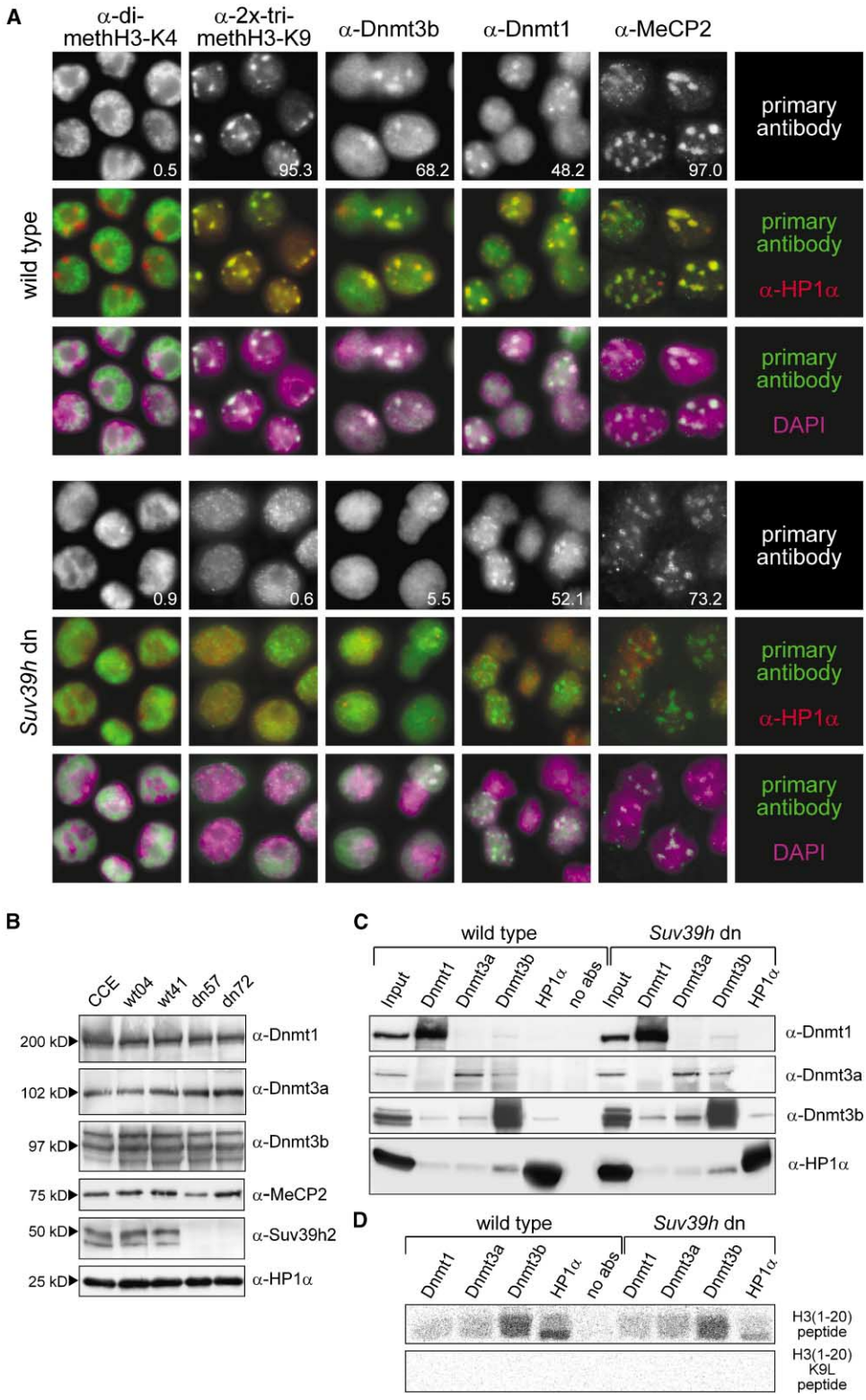

Figure 2. Pericentric Localization of Dnmt3b Is Abrogated in *Suv39h* dn ES Cells

(A) Indirect immunofluorescence of wt and *Suv39h* dn interphase chromatin in male ES cells with α-di-methH3-K4, α-2x-tri-methH3-K9, α-Dnmt3b, α-Dnmt1, and α-MeCP2 antibodies (B/W images). Colocalization with HP1α antibodies results in focal overlaps, which are indicated in yellow (merge between green and red stainings). As another means to detect accumulation at pericentric heterochromatin, costaining with DAPI was performed, resulting in the appearance of white dots (merge between green and purple stainings). Since HP1α and HP1β fail to accumulate at pericentric heterochromatin in *Suv39h* dn ES cells, costaining with DAPI was used to evaluate heterochromatic enrichment in these cells. The percentage of cells showing heterochromatic localization of the respective antibody is indicated and was evaluated by analyzing >500 nuclei in two different wt and three different *Suv39h* dn ES cell lines.

(B) Protein blots showing the abundance of the analyzed proteins in wt and *Suv39h* dn ES cells. The Suv39h2 protein is not detectable in nuclear extracts of mutant ES cells.

(C) Co-Ips, as in Figure 1B, revealing *Suv39h*-independent association of Dnmt3b with HP1α and Dnmt3a.

(D) A fluorogram showing H3-K9-specific HMTase activity associated with the various co-IPs displayed in (C).

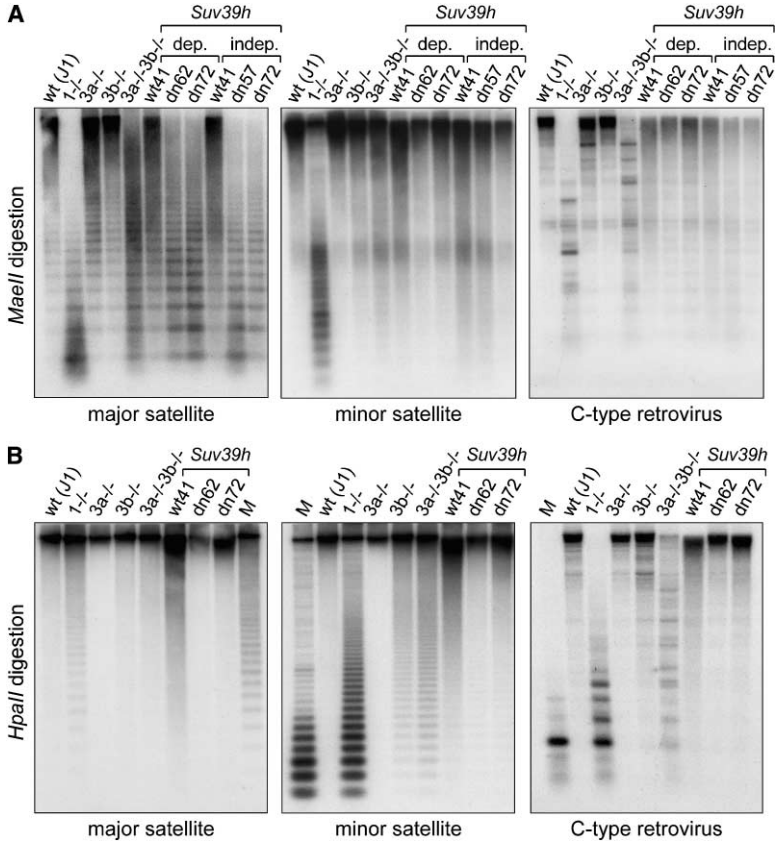

Figure 3. Impaired DNA Methylation of Major Satellite Repeats in *Suv39h* dn ES Cells

(A and B) DNA blot analyses of genomic DNA that was prepared from wt and DNMT-deficient and *Suv39h* dn ES cells and restricted with the methylation-sensitive enzymes (A) MaeII (5′-ACGT-3′) or (B) HpaII (5′-CCGG-3′). In (B), wt genomic DNA was also digested with MspI (indicated by "M"). Blots were hybridized with probes specific for major satellites (pSAT) [4], minor satellites (pMR150) [41], and endogenous C-type retroviruses (pMO) [4]. Genomic DNA from the following ES cell lines was analyzed: wt (J1), *Dnmt1*−/− (c/c) [5], *Dnmt3a*−/−, *Dnmt3b*−/−, *Dnmt3a/Dnmt3b* double deficient [3]. For the *Suv39h* double null (dn) mutants, both genomic DNA from feeder-dependent and feeder-independent ES cells was used.

termined by colabeling with DAPI-dense regions. Despite the absence of H3-K9 trimethylation, we did not observe redistribution of H3-K4 dimethylation to pericentric heterochromatin (Figure 2A, bottom panels).

Consistent with previously described pericentric accumulation [21], endogenous Dnmt3b and Dnmt1 were enriched at heterochromatic foci in 68% (Dnmt3b) and 48% (Dnmt1) of wt ES cells. The heterochromatic association of Dnmt3b, but not that of Dnmt1, was selectively impaired upon disruption of both *Suv39h* genes, and only around 5% of *Suv39h* dn ES cells retained focal Dnmt3b signals (Figure 2A, bottom panels). Since there was no significant decline in Dnmt3b expression between wt and *Suv39h* dn ES cells (Figure 2B), we conclude that the in vivo targeting of Dnmt3b to pericentric heterochromatin depends on *Suv39h*-mediated H3-K9 trimethylation and the probable adaptor function of HP1α.

To determine whether the impaired heterochromatic localization of Dnmt3b would affect DNA methylation-dependent factor recruitment, we analyzed the localization of the methyl-CpG binding protein MeCP2, which is able to interact with a single symmetrically methylated 5′-CpG in naked DNA and within chromatin [14, 19]. In extension to previous studies [22], we could detect endogenous MeCP2 at pericentric foci in >97% of wt ES cells, whereas, in *Suv39h* dn ES cells, both the frequency of MeCP2-positive cells (≈73%) and the overall intensity of pericentric MeCP2 signals was decreased (Figure 2A).

Dnmt3b Associates with HP1α in an *Suv39h*-Independent Manner

To address whether Dnmt3b also associates with HP1α in the absence of pericentric H3-K9 trimethylation, we performed co-IPs with nuclear extracts from wt and *Suv39h* dn ES cells. Whereas the Suv39h2-HP1α association was abrogated in *Suv39h*-deficient ES cell nuclear extracts (data not shown), the interaction between Dnmt3b and HP1α (Figure 2C) appeared unaltered and was also not significantly reduced if the co-IPs were performed in the presence of 20–100 μg ethidium bromide per ml (data not shown). In addition, Dnmt3a and Dnmt3b co-IP both in wt and *Suv39h* dn ES cell nuclear extracts. These data indicate that the formation of a Dnmt3a/Dnmt3b-HP1α/HP1β complex is largely independent of the presence of H3-K9 trimethylation and of HP1α and HP1β (data not shown) accumulation at pericentric heterochromatin.

Surprisingly, HMTase assays with the co-IP material showed that the H3-K9 HMTase activity associated with Dnmt3b also remained unaffected in *Suv39h* dn ES cell nuclear extracts and allowed for robust H3-K9 methylation that was comparable to levels observed with wt nuclear extracts (Figure 2D). Together with the above analyses, these data suggest that Dnmt3b participates in the formation of one or more distinct histone-DNA methylation complexes that contain an Suv39h-unrelated H3-K9 HMTase (see model below). The presence of the Suv39h enzymes, however, is required to target, via H3-K9 trimethylation, one of these putative histone-

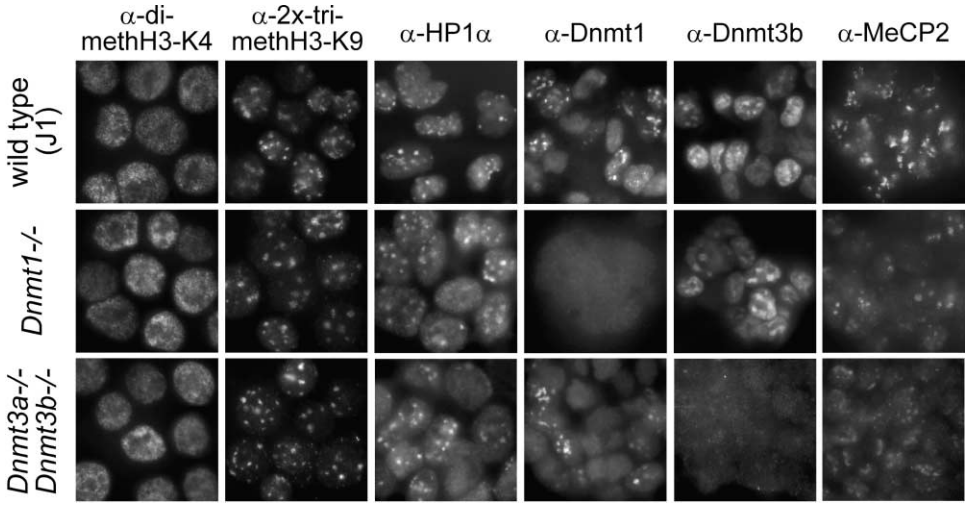

Figure 4. Pericentric H3-K9 Trimethylation Persists in DNMT-Deficient ES Cells

Indirect immunofluorescence of interphase chromatin in wt (J1), *Dnmt1*−/−, and *Dnmt3a/Dnmt3b* double-deficient ES cells as described in Figure 2A.

DNA methylation complexes to pericentric heterochromatin.

Impaired DNA Methylation at Major Satellite Repeats in *Suv39h* dn ES Cells

The aberrant in vivo localization of Dnmt3b and the reduced levels of MeCP2 at pericentric heterochromatin indicate that Dnmt3a/Dnmt3b-mediated DNA methylation patterns may be impaired in *Suv39h* dn ES cells. We therefore investigated DNA methylation at major satellite repeats and at endogenous C-type retroviruses, both of which are known targets of Dnmt3a/Dnmt3b [3]. In addition, we analyzed minor satellite repeats that, in ES cells, are de novo-methylated by Dnmt3b only [3]. Mouse major satellites consist of 1,000–10,000 copies (per chromosome) of 234 nucleotide repeat units that are localized at pericentric heterochromatin [23, 24]. In contrast, mouse minor satellites contain around 2,500 copies (per chromosome) of 123 nucleotide repeat units that are concentrated more toward the primary constriction, or centromere [23] (see model below).

We prepared genomic DNA from wt and *Suv39h* dn ES cells and, as a control, from ES cells that are mutant for the various DNMTs [3, 5]. Genomic DNA was digested with methylation-sensitive restriction enzymes, including MaeII and HpaII, and DNA blots were subsequently hybridized with major satellite, minor satellite, and C-type retroviral (Moloney-virus) probes. The results of these DNA hybridizations indicated that the absence of the Suv39h HMTases induces a selective impairment of DNA methylation at major satellites, but not at minor satellites or at endogenous C-type retroviruses (Figure 3). In contrast, DNA methylation was defective across all tested DNA sequences in ES cells lacking *Dnmt1* or cells that were double-deficient for *Dnmt3a* and *Dnmt3b*.

Reduced DNA Methylation Does Not Alter Pericentric H3-K9 Trimethylation

Next, we asked whether impaired DNA methylation would also feed back on histone methylation by examining the subnuclear distribution of H3-K9 trimethylation and HP1α localization in DNMT mutant ES cells. Comparative immunofluorescence analyses of interphase chromatin in wt (J1) versus *Dnmt1* single null or *Dnmt3a/Dnmt3b* double null ES cells indicated reduced retention of MeCP2, but no change in focal enrichment for both H3-K9 trimethylation and HP1α localization at pericentric heterochromatin (Figure 4). Likewise, we did not observe a redistribution of H3-K4 dimethylation toward pericentric heterochromatin, suggesting that the underlying major satellite repeats largely remain transcriptionally repressed in DNMT-deficient ES cells.

Transcriptional Activity across Major Satellite Repeats

Recent discoveries in *S. pombe* indicated that centromeric repeat sequences are not transcriptionally inert but can give rise to small RNA molecules that appear to be required to initiate repressive chromatin modifications, such as H3-K9 methylation [25–27]. To determine the transcriptional status of mouse major and minor satellite repeats in the wt and mutant ES cell lines, we performed RT-PCR analyses on total RNA that had been amplified with oligo-dT (Figure 5) or random primers (data not shown). For these analyses, we first isolated and sequenced several genomic clones comprising major and minor satellite repeats present in wt murine ES cells that allowed primer design for the detection of putative transcripts (Figures 5A and S2 and S3).

Using these specific primers at moderate PCR amplification (25 cycles), we detected the presence of major satellite transcripts in all wt and mutant ES cell lines. The level of these transcripts was slightly upregulated

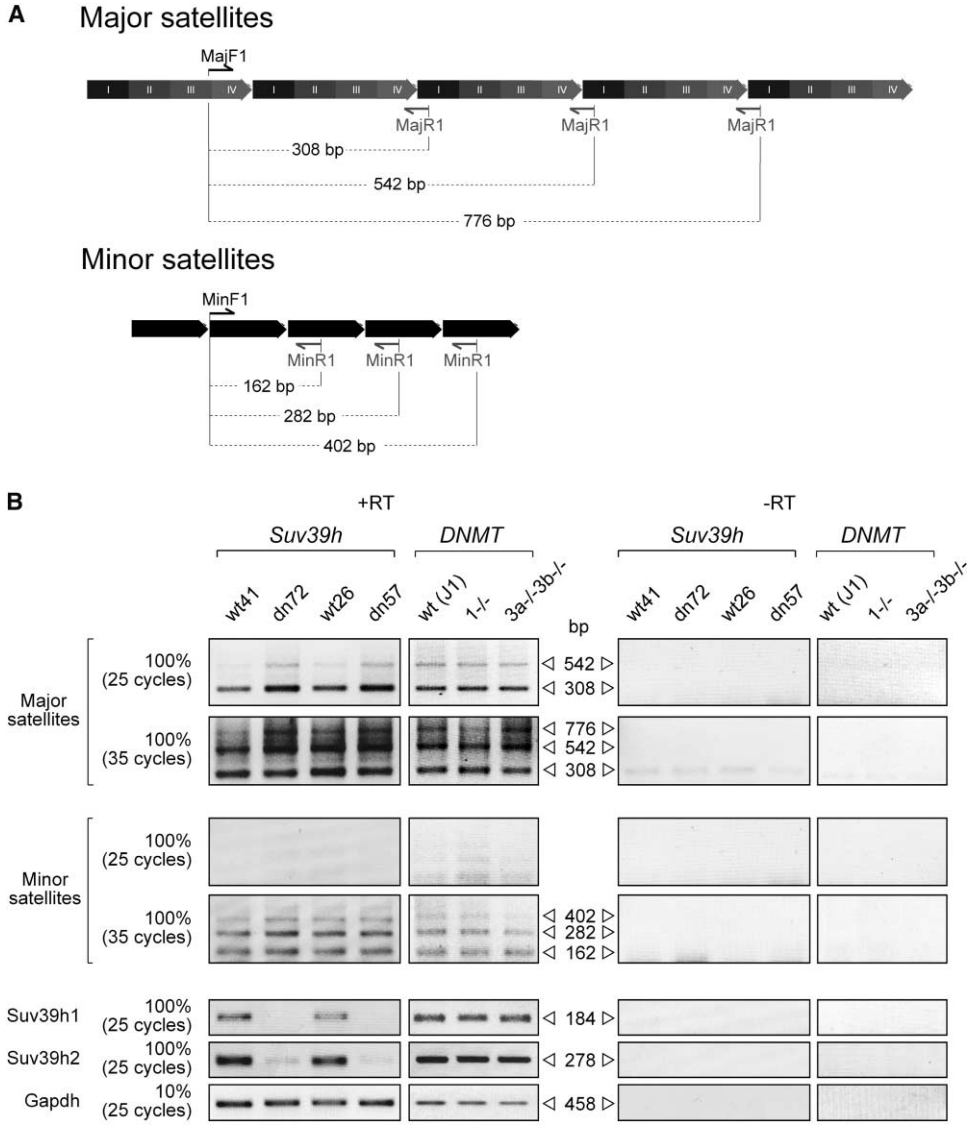

Figure 5. Transcriptional Activity across Major and Minor Satellite Repeats

(A) A schematic diagram of mouse major and minor satellite repeats indicating repeat organization and primers used to detect transcripts by RT-PCR analysis.

(B) RT-PCR analyses with oligo-dT-primed, reverse-transcribed (RT) cDNA derived from total RNA that was prepared from wt and DNMT-deficient and *Suv39h* double null (dn) ES cells. The relative amount of input RT-cDNA and the number of PCR amplification cycles using locus-specific primers (major and minor satellites, *Suv39h1*, *Suv39h2*, *Gapdh*) are indicated. As a control, reactions were performed with mock-transcribed cDNAs (−RT).

in the two *Suv39h* dn ES cell lines, but not in *Dnmt1* null or *Dnmt3a*/*Dnmt3b* double-deficient cells (Figure 5B). Using the same number of amplification cycles, we failed to reveal minor satellite transcripts, which were only detectable at higher PCR amplification (35 cycles) and which did not consistently display increased levels in *Suv39h* dn ES cells. As controls for the quality of the RT-PCR reactions, we used primers specific for *Suv39h1*, *Suv39h2*, and *Gapdh* sequences and performed the analyses in the absence of reverse transcriptase. Together, these data indicate transcriptional activity across mouse major satellite repeats and are consistent with a model in which loss of H3-K9 trimethylation and subsequent reduction in DNA methylation profiles (as is the case in *Suv39h* dn ES cells) could induce slightly higher transcript levels of repeat sequences underlying pericentric heterochromatin.

Discussion

The Suv39h HMTases Direct DNA Methylation at Pericentric Satellite Repeats

We demonstrate here by immunofluorescence analyses and DNA methylation profiles in wt and mutant murine

ES cells that Suv39h-mediated H3-K9 trimethylation can direct Dnmt3b to major satellite repeats present in pericentric heterochromatin. In addition, co-IP data suggest that Dnmt3b and Dnmt3a are part of a repressive complex that is targeted to methylated H3-K9 positions via HP1α and HP1β.

The *Suv39h*-dependent DNA methylation defect at major satellites was only detectable upon digestion with MaeII and reflects a similar deficiency in heterochromatic DNA methylation as compared to *Dnmt3a/Dnmt3b* mutant ES cells (Figure 3). In contrast, genomic DNA prepared from *Dnmt1*-deficient ES cells displayed methylation defects that were observed both after MaeII or HpaII digestion. Sequence analyses identified no apparent HpaII sites within the 234 bp major satellite repeat unit (see Figure S2) [24], suggesting that they may be interspersed between satellite repeats or present at other repetitive sequences, which together comprise the large blocks of pericentric heterochromatin. It is currently unresolved whether DNA methylation at these HpaII sites is initiated by Dnmt3a/Dnmt3b in an *Suv39h*-dependent manner and then maintained by Dnmt1, or whether there may be differential target sensitivities of DNMTs to certain DNA sequences or even to chromosomal subdomains.

In human embryonic carcinoma cell lines (Tera-1 and NCCIT), Dnmt3b also interacts with HP1α (Figure 1). Mutational inactivation of DNMT3b causes the rare ICF syndrome, which is in part characterized by extensive cytosine demethylation and chromosomal instabilities at pericentric heterochromatin containing satellite 2 and 3 repeats [28]. As human chromosomes display dense H3-K9 trimethylation at these satellites (A.H.F.M.P., submitted), we anticipate that *SUV39H*-dependent histone methylation may also direct pericentric DNA methylation in humans.

Suv39h-Independent DNA Methylation at Centromeric Satellite Repeats

In contrast to the major satellites, *Dnmt3b*-dependent DNA methylation at minor satellites is not impaired in *Suv39h* dn ES cells (Figure 3). Recent immunofluorescence and chromatin immunoprecipitation analyses with highly specific antibodies that discriminate H3-K9 di- and H3-K9 trimethylation show that the histone methylation pattern differs between centromeric and pericentric heterochromatin. For example, centromeric minor satellites are enriched for H3-K9 dimethylation in both wt and *Suv39h* dn ES cells (A.H.F.M.P., submitted), whereas pericentric major satellites display selective H3-K9 trimethylation in an *Suv39h*-dependent manner (Figure 2A). It is possible that Dnmt3b targeting to minor satellites could involve H3-K9 dimethylation, mediated by an HMTase that is distinct from the Suv39h enzymes and maintains a local concentration of HP1α or HP1β (see model, Figure 6). This interpretation would be consistent with the robust HMTase activity associated with Dnmt3b in *Suv39h*-deficient nuclear extracts (Figure 2D).

In contrast to Dnmt3b, the pericentric localization of Dnmt1 and the more complete loss of DNA methylation at major satellites observed in *Dnmt1* null versus *Suv39h* dn ES cells (Figure 3) indicates that recruitment of Dnmt1

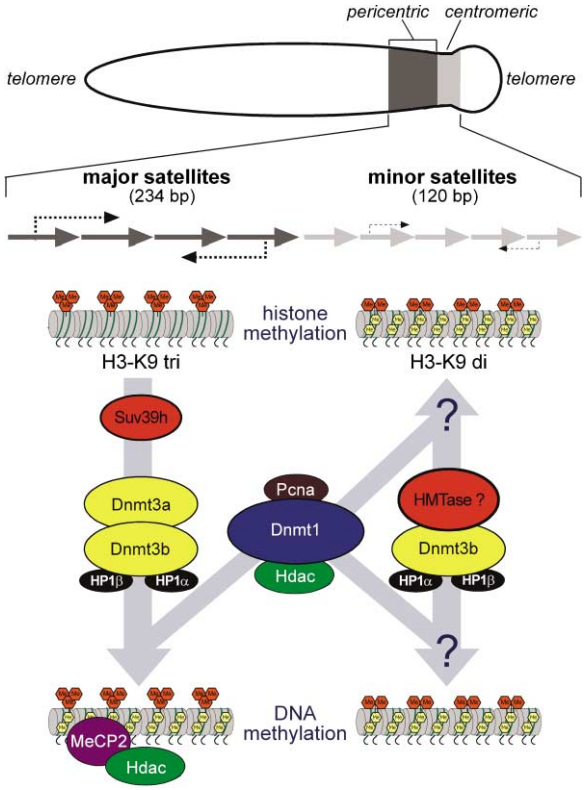

Figure 6. Histone and DNA Methylation Pathways at Mammalian Heterochromatin

The top diagram depicts the schematic localization of major and minor satellite repeats in pericentric and centromeric compartments on a mouse chromosome. Transcriptional activity across these repeats is indicated by dashed arrows. *Suv39h*-dependent H3-K9 trimethylation can direct DNA methylation at major satellites via targeting of a putative Dnmt3a/Dnmt3b-HP1α/HP1β complex. In addition, there is an *Suv39h*-independent pathway in which H3-K9 dimethylation is hypothesized to be mediated by an Suv39h-unrelated HMTase and which may interact with Dnmt3b to establish a silent chromatin domain at minor satellites. Dnmt1-mediated DNA methylation appears independent of H3-K9 methylation, but is likely to participate in the above pathways in a manner that is currently undefined. See the Discussion for a more detailed explanation.

to pericentric regions also occurs independent of the function of the Suv39h HMTases. Indeed, Dnmt1 has been shown to be targeted via PCNA to major satellites during late replication [29, 30]. Similarly, in *A. thaliana*, maintenance of CpG methylation by the *Dnmt1* homolog *MET1* is not impaired in mutants of the KYP H3-K9 HMTase [31]. These findings suggests that replication-coupled propagation of CpG methylation may be independent of H3-K9 methylation.

A Complex Interplay of Epigenetic Pathways at Pericentric Heterochromatin

Although H3-K9 methylation can be maintained at silent centromeric repeats in CpG (*met1*) or CpNpG (*cmt3*) DNA methylation-deficient mutants in *A. thaliana* [31], these studies also showed that loss of DNA methylation can feed back on the persistence of H3-K9 methylation patterns if there is significant derepression of silenced loci, e.g., observed with aberrant transcriptional activity

of retro-transposons that had integrated into pericentric domains [31]. Similarly, treatment of human cancer cell lines with the DNA-demethylating compound 5-aza-2'-deoxycytidine (5-aza-dC) results in transcriptional reactivation and reversal of repressive histone methyl marks at silenced tumor suppressor and cell cycle genes. In particular, 5-aza-dC induced a reduction in H3-K9 dimethylation while simultaneously increasing the levels for H3-K4 dimethylation and H3-K9 acetylation [32–34]. We detected by RT-PCR analysis a weak upregulation of mouse major satellite transcripts in total RNA prepared from *Suv39h* dn ES cells, but not from the different DNMT-deficient cells (Figure 5). However, pericentric heterochromatin remained underrepresented for H3-K4 dimethylation in wt and in all mutant ES cell lines examined (Figure 4). Together, these observations support a model in which reduced DNA methylation can only alter histone methylation marks if transcriptional reactivation is significantly induced [31, 35]. Since DNA methylation at major satellites is not fully lost in *Dnmt1* null or *Dnmt3a*/*Dnmt3b* double-deficient ES cells (Figure 3), it remains possible that the complete absence of DNA methylation (as e.g., in a triple-deficient *Dnmt1*/*Dnmt3a*/*Dnmt3b* ES cell line) would more drastically affect transcriptional activity and H3-K9 trimethylation patterns at the pericentric satellite repeats.

This study provides evidence that H3-K9 methylation and DNA methylation systems can synergize (see model, Figure 6) to regulate silenced chromatin domains at major and minor satellite repeats in mammals. Silencing is likely to be reinforced by binding of the methyl-CpG binding protein MeCP2 and associated histone deacetylases (HDACs) [36, 37] and HMTases [38]. The selective impairment of *Suv39h*-dependent DNA methylation at the major satellites is intriguingly reminiscent of the recently discovered potential of DNA repeats to target H3-K9 methylation to a chromatin region via the generation of small double-stranded RNAs [25–27]. Since we have observed transcripts spanning the mouse major satellites (Figure 5), it is conceivable that "small heterochromatic" RNAs generated from these transcripts may guide recruitment of the Suv39h HMTases to direct H3-K9 trimethylation and, in turn, DNA methylation to pericentric heterochromatin. Ongoing studies are aimed to delineate the molecular mechanism(s) connecting these major epigenetic pathways.

Supplemental Data

Supplemental Data including the Experimental Procedures and three supplemental figures are available at http://www.current-biology.com/cgi/content/full/13/14/1192/DC1/.

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