Pericentric Heterochromatin Generated by HP1 Protein Interaction-defective Histone Methyltransferase Suv39h1

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Background: Histone H3 lysine 9 trimethylation (H3K9me3) and heterochromatin protein HP1 accumulations are hallmarksm of heterochromatin.

Results: Pericentric accumulation of histone methyltransferase, Suv39h, and Suv39h-mediated H3K9me3 occurs without Suv39h-HP1 binding and HP1 accumulation.

Conclusion: The functional relationship between Suv39h and HP1 for pericentric heterochromatin formation is clarified.

This article has been withdrawn by the authors. In this work, we reported that pericentric accumulation of histone methyltransferase, Suv39h1 and Suv39h1-mediated H3K9me3 can take place in the absence of the interaction of Suv39h1 with HP1 and HP1 accumulation. In extending this work, we found that we could not reproduce our finding that there was pericentric accumulation of the HP1 interaction-defective Suv39h1 mutant (Suv39h1 1-41 deletion mutant) in Suv39h-deficient ES cells (depicted in Fig. 2, C and G); we again observed induction of pericentric H3K9me3. Although we could not repeat precisely the same experiments as the supply of the original cell lines had been exhausted, our recent results no longer support our original published conclusions. Therefore, we wish to withdraw this paper. We apologize for any inconvenience that may have resulted from its publication.

Chromatin exists in two forms, euchromatin and heterochromatin (1). Euchromatin is the loosely packed form of chromatin that is rich in gene concentration and often undergoes active transcription. In contrast, heterochromatin is tightly packed and is in the transcriptionally repressed state. The pericentromere is a heterochromatic domain that provides a structural function for chromosome (2). Therefore, at heterochromatin, various transcriptionally silent effector molecules are recruited by the heterochromatin-specific epigenetic marks. Heterochromatin protein 1 (HP1) is such an effector molecule that was originally discovered in Drosophila as a dominant suppressor of position-effect variegation (9). Similar to Suv39h, the HP1 family is evolutionarily conserved, with members in fungi, plants, and animals, and it has multiple isoforms within the same species (10).

The N-terminal chromodomain (CD) shows a high affinity to methylated H3K9 (highest affinity for H3K9me3), causing HP1 to be tethered to heterochromatin (11, 12). This recruitment system is also highly conserved in different species. Furthermore, this regulation is interdependent. For example, the HP1...
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homolog Swi6 in fission yeast is also crucial for the Suv39h homolog Clr4 accumulation and Clr4-mediated H3K9 methylation to heterochromatin (13). HP1 homologs can physically interact with Suv39h homologs in different species and sequential cycles of Swi6 binding, and Clr4 recruitment/deposition of H3K9me have been proposed (14) to explain the interdependent regulation of Clr4- and Swi6-mediated silent heterochromatin formation.

A similar functional concept has been proposed for the Suv39h- and HP1-mediated heterochromatin formation in mammals (11, 12). However, so far, it has not been validated experimentally much whether HP1 binding to Suv39h is crucial for Suv39h-mediated heterochromatin formation, which is what we are addressing in this study.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—DNA fragments encoding FLAG-tagged Suv39h1, catalytically dead mutant Suv39h1 H324L (H324L), and N-terminally truncated mutant Suv39h1 Δ(1-41) (ΔN) were inserted into the pCAG-iresPuro vector. DNA fragments encoding Myc-tagged mouse HP1α, HP1β, and HP1γ were inserted into the pcDNA3.1 vector.

**Antibodies**—Antibodies used for Western blotting, immunoprecipitation (IP), immunofluorescence, and chromatin immunoprecipitation (ChIP) analyses are as follows: anti-H3K9me3 (2F3 (15)); anti-H4K20me3 (27F10 (16)); anti-FLAG (WAKO, 018-22381) for immunofluorescence; anti-HP1α (Santa Cruz Biotechnology, sc-52921), and anti-Dnmt3b (2F3 (15)); anti-H4K20me3 (27F10 (16)); anti-FLAG (1E6, WAKO, 018-22381) for immunofluorescence; anti-HP1α (Santa Cruz Biotechnology, sc-52921), anti-HP1β (Santa Cruz Biotechnology, sc-560688), and anti-HP1γ (Santa Cruz Biotechnology, sc-560700) for Western blotting (Millipore, MAB3584); anti-ATRX (Santa Cruz Biotechnology, sc-60111); anti-Dnmt1 (Santa Cruz Biotechnology, sc-52757); anti-Dnmt3a (Santa Cruz Biotechnology, sc-373744); anti-Dnmt3b (IMAGEs, IMG-184 A).

**Protein Immunoprecipitation**—48 h after electroporation, HEK293 T cells were incubated in PBS containing 5 mM dimethyl dithiobispropionimidate at 4 °C for 1 h. Whole cell lysate was obtained using lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.3% digitonin, 20 mM N-ethylmaleimide) after quenching for 10 min at 4 °C using 150 mM glycine PBS. We then followed two protocols. 1) The FLAG-IP protocol wherein the lysate was incubated with an anti-FLAG antibody affinity gel (Sigma, F4799). 2) The MYC-IP protocol wherein the lysate was incubated with anti-Myc (9E10) for 2 h at 4 °C. The immune complex was washed with washing buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.1% digitonin) three times, and then precipitated proteins were eluted by an excess amount of 3× FLAG peptide (Sigma, F4799). 2) In the myc-IP protocol, the lysate was incubated with anti-Myc (9E10) for 2 h at 4 °C. The immune complex was captured using protein G-Sepharose (GE Healthcare, 17-0618-02) and washed with washing buffer. For Western blot analysis, anti-FLAG (M2) and anti-Myc (9E10) were used as primary antibodies, and HRP-conjugated anti-mouse Ig (RLK, 18-8817-31) was used as a secondary antibody.

**Cell Culture**—ES cells were maintained in Dulbecco’s modified Eagle’s medium (Sigma) containing 15% fetal calf serum, leukemia-inhibiting factor, penicillin/streptomycin, 1-glutamine, nonessential amino acids, and β-mercaptoethanol (ES medium). To generate ES cells stably expressing FLAG-tagged Suv39h1, H324L, and ΔN, their expression vectors described above were introduced into Suv39h dn ES cells (SDK (17)) or Suv39h dn (6) (ES cell line) via electroporation system (Bio-Rad Gene Pulser Xcell). Stable expression clones were selected in ES medium containing puromycin (1 μg ml⁻¹).

**Immunofluorescence Analysis**—Cells cultured on chamber slides (nunc, 177437) were fixed with 4% paraformaldehyde for 8 min at room temperature, permeabilized with 1% Triton X-100 for 15 min, and incubated overnight with primary antibodies (4 °C). Anti-mouse IgG conjugated with Alexa-568,488 Fluor (invitrogen) was used as a secondary antibody. The nuclei were counterstained with DAPI, as observed under a confocal microscope (Olympus, FV1000). Three-dimensional reconstructed image analysis was performed using the Z-stack function of analysis software (FV10 ASW). Signal intensity of the antigen per DAPI intensity was measured by ImageJ.

**ChIP Analysis**—ES cells (5 × 10⁶) were cross-linked in 1 ml of 5% formaldehyde in DMEM for 5 min at room temperature. After removing formaldehyde solution, cells were incubated with 1 mM glycine PBS for 10 min. Cells washed by PBS were incubated in RIPA buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate) for 10 min at room temperature. After centrifugation (13,000 rpm for 10 min), the supernatant (500 μl) was collected and combined with an additional 500 μl of ChIP dilution buffer. Then 300 μl of the lysate mixture was further diluted with 200 μl of 1 × RIPA buffer 150 mM (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate) was added, and the samples were sonicated with Bioruptor (Cosmo Bio, 150W, power high, 15 s on and 30 s off for 24 cycles). After centrifugation (13,000 rpm for 10 min), the supernatant (500 μl) was collected and combined with an additional 500 μl of ChIP dilution buffer. Then 300 μl of the lysate mixture was further diluted with 200 μl of 1 × RIPA buffer 150 mM (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate), incubated with primary antibody overnight at 4 °C, and then incubated for further 4 h after addition of 20 μl of Dynabeads conjugated with anti-mouse IgG (Invitrogen, 112.01D). The immune complexes were sequentially washed in 1 × RIPA buffer 150 mM, 1 × RIPA buffer 500 mM (50 mM Tris-HCl, 500 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate), 1 × TE buffer. After de-cross-linking by incubation in ChIP direct elution buffer (10 mM Tris-HCl, 300 mM NaCl, 5 mM EDTA, 0.5% SDS) at 65 °C overnight, DNA was isolated by phenol/chloroform extraction following proteinase K treatment (0.1 mg/ml) of the immune complex for 60 min at 56 °C and then analyzed by real time PCR using primers specific for major satellite as reported (16).

**Southern Blot Analysis with Methylation-sensitive Restriction Enzyme**—Genomic DNA was isolated and digested with methylation-sensitive restriction enzyme Mael and analyzed on DNA blot. Major satellite repeat DNA probe was amplified using primers reported for major satellite as reported (16).

**Enzyme**—DNA fragments encoding FLAG-tagged Suv39h1, catalytically dead mutant Suv39h1 H324L, and ΔN, their expression vectors described above were introduced into Suv39h dn ES cells (SDK (17)) or Suv39h dn (6) (ES cell line) via electroporation system (Bio-Rad Gene Pulser Xcell). Stable expression clones were selected in ES medium containing puromycin (1 μg ml⁻¹).
RESULTS

N-terminal Region of Mouse Suv39h1 Was Essential for HP1 Interaction—It has been shown that the N terminus of Suv39h1 and *Drosophila* homolog Su(var)3-9, which is upstream of the CD, interacts with the chromoshadow domain (CSD) of HP1 *in vitro* (18–20). Therefore, we assayed the interaction of wild-type (WT) mouse Suv39h1 and the N-terminal deletion (Δ1–41) mutant (named ΔN) with mouse HP1α, -β, and -γ in HEK293T cells (Fig. 1). As shown in Fig. 1B, Myc-tagged HP1α, -β, and -γ were clearly co-immunoprecipitated with FLAG-tagged Suv39h1 but not with ΔN (center IP:FLAG, two panels). Anti-Myc co-immunoprecipitation experiments showed the same results (Fig. 1B, bottom IP:myc, two panels). These data demonstrate that the ΔN mutant of Suv39h1 does not interact with HP1.

Pericentric Focus Formation of ΔN-expressing H3K9me3 Could Be Induced in the Suv39h1dn ES Cells, but HP1 Accumulation Is Impaired—To address how essential for Suv39h-mediated epigenetic heterochromatin formation, we introduced WT Suv39h1 (Suv39h1 WT), ΔN-expressing Suv39h1 (ΔN), and Suv39h1ΔN (ΔN) ES cells (6, 17). It is also known that expression of Suv39h1 cannot establish/maintain H3K9me3 and HP1 focus formation on pericentric regions in a Suv39h1-deficient background (12). Therefore, we introduced the same enzymatically inactive mutant of Suv39h1 (named H324L, Fig. 1A). Immunofluorescence staining analysis clearly showed that there is no accumulation of H3K9me3, HP1α, -β, and -γ on pericentric DAPI-dense regions in the *Suv39h1* dn ES cells (Fig. 2, A, B, and D) as reported previously (12). Introduction of FLAG-tagged WT Suv39h1, but not H324L, rescued H3K9me3 and HP1α, -β, and -γ focus formation on DAPI-dense regions in the *Suv39h1* dn ES cells. Interestingly, these H3K9me3 signals were also recovered by the expression of FLAG-tagged ΔN; however, pericentric HP1α, -β, and -γ signals were still rarely detectable (Fig. 2, A, B, and D). Furthermore, FLAG-tagged ΔN in the *Suv39h1* dn ES cells was accumulated on the DAPI-dense regions, as shown for WT Suv39h1 (Fig. 2C) (12). Western blot analysis showed that the expression of HP1α, -β, and -γ was not changed in these transfected cells (Fig. 2E), suggesting that the level of HP1 protein was not affected. Accumulation of H3K9me3 and FLAG-tagged Suv39h1 (WT and ΔN) on the pericentric regions was also validated by ChIP-quantitative PCR analysis. Fig. 2, F and G, clearly shows that H3K9me3 on the pericentric major satellite repeat regions was rescued by not only WT Suv39h1 but also ΔN; both molecules were enriched on these loci. Furthermore, it was also confirmed by HP1β ChIP-quantitative PCR analysis that HP1β accumulation is marginally restored by ΔN expression (Fig. 2F). Because all these phenotypes were observed in multiple stable expressing clones for each construct, we have shown the results for only one of the clones (i.e. representative clone data). Moreover, immunofluorescence staining data (low magnification images) for only two clones are shown (Figs. 2, I and J, 3, F and G, and 5, F and G).

In conclusion, these results show that *Suv39h1*ΔN, which does not interact with HP1, can be recruited to and accumulate in the pericentric regions and deposit H3K9me3. HP1 possesses binding activity with methylated H3K9; however, this activity was not sufficient for substantive HP1 pericentric accumulation without Suv39h binding.

**H4K20me3 and ATRX Did Not Accumulate at Pericentric Heterochromatin in the *Suv39h1*ΔN ES Cells Expressing ΔN**—In addition to H3K9me3 and HP1, other epigenetic heterochro-
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A

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C

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Z
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Immunohistochemical staining analysis for H3K9me3 (red) and HP1α (green) (A), HP1α (red) and HP1γ (green) (B), or FLAG-tagged Suv39h1 WT, H324L, or ∆N (red) (C) was conducted with WT or Suv39h1 DN ES cells or Suv39h1 DN ES cells complemented with either FLAG-tagged WT Suv39h1, H324L, or ∆N. DNA was counterstained with DAPI (blue). DAPI intensity and the intensity of the antigen at the same region were quantified using ImageJ based on the fluorescence imaging data obtained for A–C. The intensities for 30 cells were quantified. The value of each antigen intensity was expressed as a ratio between the DAPI intensity and the intensity of the antigen being measured. E. Western blot analysis of endogenous HP1 subtypes in the indicated cell lines. F. H4K20me3 chromatin was isolated from the indicated cell lines. CHIP-quantitative PCR analysis was conducted using anti-H3K9me3 (F), anti-FLAG (G), anti-HP1B (H), and primers specific for major satellite repeats and the Gapdh gene. I and J, Immunohistochemical staining analysis for the two independent cell lines indicated at low magnification. A combination of color and antigen was same as that observed in A and B.
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A

B

C

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F

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WITHDRAWN
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In the Suv39h dn ES cells, H3K9me3 and other repressive epigenetic molecules or marks such as HP1, ATRX, Suv4-20h, Dnmt3a and -3b, H4K20me3, and DNA methylation were depleted or reduced from pericentric DAPI-dense regions. However, major satellite repeats were derepressed. In this study, we demonstrated that the Suv39h1 mutant that did not interact with HP1 (\( \Delta N \)) could rescue the pericentric accumulation of itself and H3K9me3 deposition. Furthermore, Dnmt3a, -3b, and DNA methylation were recovered on the pericentric regions. However, pericentric accumulation of HP1 was severely affected, and ATRX and H4k20me3 were not recovered at all. These phenotypes are shown in Fig. 7.

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DISCUSSION

In the Suv39h dn ES cells, H3K9me3 and other repressive epigenetic molecules or marks such as HP1, ATRX, Suv4-20h, Dnmt3a and -3b, H4K20me3, and DNA methylation were depleted or reduced from pericentric DAPI-dense regions. However, major satellite repeats were derepressed. In this study, we demonstrated that the Suv39h1 mutant that did not interact with HP1 (\( \Delta N \)) could rescue the pericentric accumulation of itself and H3K9me3 deposition. Furthermore, Dnmt3a, -3b, and DNA methylation were recovered on the pericentric regions. However, pericentric accumulation of HP1 was severely affected, and ATRX and H4k20me3 were not recovered at all. These phenotypes are shown in Fig. 7.

HP1 Binding to Suv39h1 Was Dispensable for the Establishment of Suv39h1 Pericentromere Accumulation and H3K9me3 Formation—It is known that HP1 homolog Swi6 is crucial for Clr4-mediated H3K9 methylation of heterochromatin in fission yeast (13). Furthermore, it has been proposed that the physical interaction of Clr4 with Swi6 is important for the establishment/spreading of H3K9 methylation (30). A similar functional concept for the Suv39h-HP1 complex on the H3K9me-mediated heterochromatin establishment/spreading has been proposed for other species, including mammals (11, 12). However, the analysis of Suv39h dn ES cells rescued with \( \Delta N \) demonstrated that HP1 binding to Suv39h was dispensable for the establishment of Suv39h1 pericentromere accumulation.
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A

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and its H3K9me3 formation. The level of H3K9me3 over the major satellite repeat regions in the Suv39h dn ES cells was completely recovered by ΔN, similar to that in WT ES cells (Fig. 2F). These findings are consistent with a previous report indicating that the recruitment of SUV39H1 to heterochromatin is at least partly independent from HP1 interaction (31). However, this does not exclude the possibility that heterochromatic regions are not fully reconstituted in the absence of HP1 at the heterochromatin-euchromatic boundaries or facultative heterochromatins in euchromatin. Future studies will examine the Suv39h recruitment/accumulation mechanism from this perspective.

Critical Role of Suv39h on HP1 Pericentromere Accumulation—It has been well established that Suv39h/Su(var)3-9/Cld4 is essential for HP1/Swi6 heterochromatin accumulation (12, 18, 35). However, this HP1 heterochromatin accumulation is induced/maintained by (at least) two distinct mechanisms, one of which is HP1 CD-mediated and the other is CSD-mediated. In case of mouse HP1β, both the binding activity of CD to methylated H3K9 and the binding site of CSD to the PXVXL-containing proteins are crucial for HP1β pericentromere accumulation (36). In other artificial experiments, the Gal4-SUV39H1 fusion tethering system showed that H3K9 methylation is not sufficient for recruitment of HP1 to chromatin, but the direct interaction of HP1 with SUV39H1 is also important (37). Furthermore, both H3K9me binding and CSD binding to PXVXL-containing proteins are important for nucleosome binding of Drosophila HP1 in vitro (20). In this case, HP1 can bind to Su(var)3-9 through the PXVXL-containing protein-binding site, and HP1 nucleosome binding is enhanced by Su(var)3-9 loading. Because this enhancement is blocked by the
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**A**

WT

HP1

Suv39h

ATRX

H3K9me1

H3K9me2

H4K20me3

mCG

**B**

Suv39h dn

major satellite repeat transcript

**C**

Suv39h dn expressing ΔN

HP1

Dnmt3a/b

**FIGURE 7. Epigenetic landscape of mouse pericentromeric heterochromatin regulated by Suv39h.** A, in wild-type mouse ES cells, Suv39h localize to pericentromere regions dependent on H3K9me3 and repression of major satellite repeats. HP1 also accumulated on pericentromere regions dependent on the Suv39h interaction and H3K9me3. Recruitment of other pericentromere proteins, such as Suv39h and ATRX, to the pericentromere regions dependent on HP1 or the H3K4me0/K9me3 interaction, respectively. The recruited Suv39h and ATRX add H4K20me3. Suv39h also recruited Dnmt3a/b to the pericentromere and induced DNA methylation of major satellite repeats. The epigenetically organized structure was crucial for functionally silent heterochromatin and repression of major satellite repeats. B, in the Suv39h dn ES cells, HP1, Suv4-20h, ATRX, and Dnmt3a/b were depleted from the pericentromere due to deficiency of Suv39h and Suv39h-mediated H3K9me3. Because Suv4-20h and Dnmt3a were depleted from the pericentromere, H4K20me3 and DNA methylation were severely reduced. Major satellite repeats were derepressed. C, in the Suv39h dn ES cells expressing Suv39h1 ΔN, ΔN localized to the pericentromere and deposited H3K9me3. However, HP1 pericentromere accumulation was generally not recovered even though H3K9me3 was restored because the HP1-Suv39h interaction was missing. Suv4-20h, H4K20me3, and ATRX were not restored on the pericentromere likely due to a lack of HP1 pericentromere accumulation. However, ΔN recruited Dnmt3a/b causing DNA methylation. This incomplete heterochromatin could partially silence transcription of major satellite repeats.

Disruption of the HP1-Su(var)3-9 interaction, it is proposed that the CSD-mediated HP1 heterochromatin accumulation was generally not restored; if the level of WT and Suv39dn were defined as 100 and 0%, respectively, only ~10–20% of the WT level for HP1α, -β, and -γ signals on the DAPI-dense regions were detected in Suv39h dn cells expressing ΔN (Fig. 2D). However, such a severely depleted phenotype is only induced by CD and CSD dual mutations and not by each single mutation in the HP1β studies (36). Thus, if H3K9me3 is restored in the Suv39h dn cells expressing ΔN, one might expect that the HP1 pericentromere accumulation would be partially affected. Our current hypothesis for this ΔN phenotype is as follows. Because in the Suv39h dn cells expressing ΔN, the HP1 CSD-Suv39h module is not functional, and other HP1-interacting heterochromatin molecules such as ATRX and Suv4-20h are also absent from the pericentric regions, these HP1-binding molecules may also contribute to HP1 pericentromere accumulation. Therefore, in the ΔN case, even though the HP1-H3K9me binding module is functional, several other HP1-binding heterochromatin molecules are missing, which may be the reason for the severe HP1 loss of phenotype.

*ATRX Pericentromeric Heterochromatin Accumulation*—In addition to HP1, Suv39h pericentromeric heterochromatin accumulation is also maintained by methyl-CpG binding protein 2 (MeCP2) (35, 36). In wild-type mouse ES cells, MeCP2 expression is ~20% of the WT level for HP1 and HP1 is not localized at the pericentromere (23, 24). In Suv39h dn ES cells, MeCP2 expression is ~20% of the WT level for HP1 and HP1 is not localized at the pericentromere (23, 24). This suggests that partial HP1 recruitment to pericentromeres is sufficient to recruit MeCP2, which is consistent with previous observations that HP1 binds to ATRX through the HP1 H3K9me3 binding module (37). Therefore, inactivation of each signaling module has a partial or small impact on ATRX and MeCP2 recruitment to pericentromeric heterochromatin (23, 24). In ATRX-null cells, all these modules are inactivated; thus 1) the ATRX binding module is not functional because pericentric H3K9me3 is highly suppressed; 2) the HP1-mediated recruitment is absent because HP1 is not localized at the pericentromere, and 3) MeCP2-mediated recruitment is absent because MeCP2 is undetectable in ES cells. Although H3K9me3 formation is recovered by ΔN, the two modules are not functional. Therefore, it is not surprising that the ATRX phenotype is not rescued at all in the Suv39h dn cells expressing ΔN.

*DNA Methylation of Major Satellite Repeats*—ΔN could not rescue HP1 or the HP1-interacting heterochromatin molecules to pericentromeric localization, but DNA methylation of major satellite repeats was rescued to the level of WT ES cells and the Suv39h dn ES cells rescued by WT Su39h1 (Fig. 5A). Two possible mechanisms of Suv39h-mediated DNA methylation have been proposed. One is the H3K9me3-mediated indirect recruitment mechanism because HP1 binds to Dnmt3b (6). The other one is the Suv39h direct mechanism because Suv39h1 can bind to Dnmt3a (38). Our data suggest the latter mechanism because WT Suv39h1 and ΔN could rescue Dnmt3a and -3b, but not HP1 (data not shown) pericentromere recruitment in the Suv39h dn cells (Fig. 5, B–D).

Although DNA methylation of major satellite repeats in the Suv39h dn ES cells expressing ΔN was rescued to the WT ES level, derepressed major satellite repeat were only partially suppressed. In contrast, WT Suv39h1 could completely suppress this derepression. It is known that HP1 and ATRX depleted from the pericentromeres play a role in transcriptional silencing (9, 39–43). Our new experimental evidence further indi-
icates that Suv39h is the major master regulator of heterochromatin formation. Thus, it recruits multiple downstream molecules to the pericentric regions and creates a functional heterochromatin structure using several mechanisms such as a direct interaction or a H3K9me-mediated interaction.

In conclusion, we have shown that the HP1 interaction with Suv39h or HP1 pericentromere accumulation is mostly dispensable for Suv39h accumulation and H3K9me3 formation at pericentric regions. However, the mechanism of Suv39h or H3K9me3 target specificity to the pericentromere still remains unknown. We hope our new findings will provide some insights to understand this challenging and long-standing problem.

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