REVIEW

H3K9 methyltransferase G9a and the related molecule GLP

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The discovery of Suv39h1, the first SET domain-containing histone lysine methyltransferase (HKMT), was reported in 2000. Since then, research on histone methylation has progressed rapidly. Among the identified HKMTs in mammals, G9a and GLP are the primary enzymes for mono- and dimethylation at Lys 9 of histone H3 (H3K9me1 and H3K9me2), and exist predominantly as a G9a–GLP heteromeric complex that appears to be a functional H3K9 methyltransferase in vivo. Recently, many important studies have reported that G9a and GLP play critical roles in various biological processes. The physiological relevance of G9a/GLP-mediated epigenetic gene regulation is discussed.

The role of histone post-translational modifications, especially histone lysine methylation, in diverse biological processes has been a rapidly growing research subject in the last decade. Since the Su(var)3-9–Enhancer of zeste–Trithorax (SET) domain of Suv39h1/KMT1a was demonstrated to be the catalytic domain for lysine methylation (Rea et al. 2000), many SET domain-containing molecules have been found to be novel histone lysine methyltransferases (HKMTs) in various species, including viruses, bacteria, yeast, fungi, and other multicellular organisms (Qian and Zhou 2006; Pontvianne et al. 2010). With the sole exception of Dot1/Dot1L, which methylates Lys 79 of histone H3, all of the identified HKMTs are SET domain-containing molecules. Furthermore, since two classes of histone lysine demethylases (HKDMs) have been discovered (Shi et al. 2004; Tsukada et al. 2006), it is now apparent that methylation of histone lysine residues is dynamically regulated by specific HKMTs and HKDMs (Klose and Zhang 2007; Mosammaparast and Shi 2010). Lysine demethylase 1 (LSD1, also known as KDM1) is a flavin adenine dinucleotide-containing enzyme that removes mono/dimethylation. The Jumonji C-terminal domain (JmjC) family of HKDMs uses Fe^{2+} and α-ketoglutarate as cofactors to remove all methylation states. In addition to histones, nonhistone substrates also have been reported for various HKMTs (SET7/SET9, G9a, GLP, PR-SET7/SET8, SMYD2, and SMYD3) (Sampath et al. 2007; Huang and Berger

2008) and HKDMs (LSD1) (Nicholson and Chen 2009). The biological significance of nonhistone lysine methylation currently is not well understood, and is also a subject of growing interest.

G9a/KMT1C and GLP/Eu-HMTase1/KMT1D are members of the Suv39h subgroup of SET domain-containing molecules, and together they are the key HKMTs for H3K9me1 and H3K9me2 (Jenuwein et al. 1998; Tachibana et al. 2001, 2002, 2005). Among the mammalian HKMTs, G9a and GLP have been extensively investigated at the biochemical and physiological levels, and some specific inhibitors have been developed. Recently, multiple studies have identified the critical roles that G9a and GLP play in diverse biological processes. This review introduces the biochemical properties of G9a and GLP and specific inhibitors, describes the biological significance of these enzymes, and discusses the unresolved aspects of G9a/GLP-mediated epigenetic gene regulation.

Biochemical properties of G9a and GLP

Lysine methyltransferase activity

G9a was reported as the second HKMT and can methylate histone H1 and H3 (K9 and K27) in vitro (Tachibana et al. 2001). Biochemically, both Suv39h1 and G9a can catalyze mono-, di-, and trimethylation reactions on H3K9 (Collins et al. 2005; Kubicek et al. 2007). Investigations of G9a-deficient and Suv39h1/h2-double-deficient cells demonstrated that Suv39h1/h2 are crucial HKMTs for H3K9me3 on pericentromeric heterochromatin, and that G9a is a major H3K9me1 and H3K9me2 HKMT of euchromatin (Tachibana et al. 2002; Peters et al. 2003; Rice et al. 2003). G9a is also involved in H3K9me3 modification in vivo (Yokochi et al. 2009), and it has been shown recently that G9a is indeed involved in H1 and H3K27 methylation in vivo (Trojer et al. 2009; Weiss et al. 2010; Wu et al. 2011).

GLP was originally described as a gene encoding a G9a-like protein. Since then, various biochemical studies have demonstrated that GLP and G9a possess the same substrate specificities on histones (Ogawa et al. 2002; Tachibana et al. 2005; Weiss et al. 2010). Although G9a and GLP independently exert HKMT activities and have the same histone substrate specificities in vitro, the levels of H3K9me1 and H3K9me2 are severely reduced by either G9a or GLP knockout. Moreover, the G9a and GLP double knockout

does not further reduce H3K9me1 and H3K9me2 levels (Tachibana et al. 2005). Thus, G9a generally cannot compensate for the loss of GLP HKMT function in vivo, and vice versa.

Biochemical characterization of G9a and GLP showed that G9a and GLP can form a homomeric and heteromeric complex via their SET domains; however, endogenous molecules exist exclusively as the stoichiometric G9a–GLP heteromeric complex in various human and mouse cells (Tachibana et al. 2005). Therefore, the G9a–GLP heteromeric complex seems to be the functional H3K9 HKMT in vivo (Tachibana et al. 2005, 2008). Furthermore, complementation experiments using methyltransferase-defective mutants have shown that the enzymatic activity of G9a is more important for the in vivo HKMT function than that of GLP (Tachibana et al. 2008). Although it is not yet clear why G9a or GLP generally cannot function alone as an HKMT in vivo, there are some possible mechanistic explanations for the dominant existence of the G9a–GLP heterocomplex inside cells, even though G9a and GLP can also form homodimers. First, G9a forms a stoichiometric complex not only with GLP but also with Wiz, a multi-zinc finger-containing molecule. Knockout or knockdown of *GLP* or *Wiz* concomitantly reduces G9a protein levels (Tachibana et al. 2005; Ueda et al. 2006). Second, Wiz seems to recognize and bind the homodimer or heterodimer structure of G9a and GLP SET domains, but most stably interacts with the G9a–GLP heterodimer (Ueda et al. 2006). Therefore, we speculate that the G9a–GLP(–Wiz) complex is the most stable form, and therefore exists as the dominant intracellular form. The domain organization of the core G9a complex components G9a, GLP, and Wiz is shown in Figure 1.

Link between H3K9 methylation and DNA methylation

In *Neurospora*, H3K9 methylation controls DNA methylation (Tamaru and Selker 2001; Rountree and Selker 2010). The DNA methyltransferase DIM-2 forms a complex with HP1. The DIM-5 HKMT methylates H3K9 to form H3K9me3. Subsequently, the DIM-2–HP1 complex

is recruited to H3K9me3-positive nucleosome loci via HP1 binding to H3K9me3 and induces DNA methylation. In *Arabidopsis*, DNA methylation and H3K9 methylation are functionally interdependent (Jackson et al. 2002; Malagnac et al. 2002; Johnson et al. 2007; Tamaru 2010). The SET- or RING-associated (SRA) domain of the HKMT KRYPTONITE (KYP) binds to methylated DNA. In effect, KYP targets nucleosomes on methylated DNA and methylates H3K9 to form H3K9me2. Reciprocally, H3K9me2 recruits the DNA methyltransferase CHROMOMETHYLASE 3 to induce DNA methylation.

There are functional links between H3K9 methylation and DNA methylation in mammals as well. DNA methylation at major satellite repeats is reduced in *Suv39h1* and *Suv39h2* double-knockout embryonic stem (ES) cells (Lehnertz et al. 2003). Since the pericentromeric heterochromatin recruitment of HP1α and HP1β depends on Suv39h-mediated H3K9 methylation, and since HP1α and HP1β interact with DNA methyltransferase 3b (Dnmt3b), functional roles for Suv39h-mediated H3K9 methylation and HP1 recruitment in the regulation of DNA methylation have been proposed (Lehnertz et al. 2003).

DNA methylation is also affected in *G9a* or *GLP* knockout mouse ES cells (Dong et al. 2008; Epsztejn-Litman et al. 2008; Tachibana et al. 2008). However, in this case, this G9a/GLP-dependent DNA methylation is independent of the in vivo histone methyltransferase activity; i.e., catalytically inactive G9a partially restored the aberrant DNA methylation pattern in G9a$^{-/-}$ cells. There are some reports that DNMT1 regulates H3K9 methylation, although this matter is still open for debate. In HeLa cells, DNMT1 interacts with G9a, regulating chromatin loading of G9a, and knockdown of *DNMT1* induces a reduction in H3K9me2 levels (Estève et al. 2006). Furthermore, *DNMT1* knockout HCT116 human colon cancer cells also show a reduction in H3K9me2 and H3K9me3 levels (Espada et al. 2004). However, there is no reduction of H3K9me2 in *Dnmt1* knockout or *Dnmt1*, *Dnmt3a*, and *Dnmt3b* triple-knockout mouse ES cells (Tsumura et al. 2006).

The hemimethylated DNA-binding molecule UHRF1/ICBT90/NP95 forms a complex with Dnmt1 and mediates
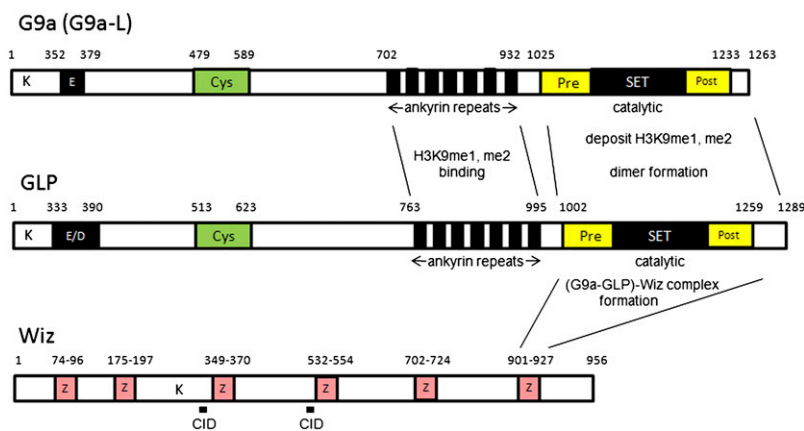

Figure 1. Domain organization of the core G9a complex components: G9a, GLP, and Wiz. Amino acid sequences of mouse (m) G9a-L (9QZ148), mGLP (A2AIS4), and mWiz (O88286-2). (K) Potential methylation sites by G9a or GLP (K167 and K239 in G9a-L, K154 and K206 in GLP, and K467 in Wiz) (Sampath et al. 2007; Rathert et al. 2008); (E) Glu-rich region; (E/D) Glu/Asp-rich region; (Cys) Cys-rich region; (Pre) pre-SET domain; (SET) SET domain; (Post) post-SET domain; (Z) zinc finger motif; (CID) CtBP-interacting region (Ueda et al. 2006).

Dnmt1-mediated maintenance DNA methylation during replication (Bostick et al. 2007; Sharif et al. 2007). Furthermore, the tandem Tudor domain and the plant homeodomain (PHD) of UHRF1 bind H3K9me2 and H3K9me3 (Hashimoto et al. 2009; Karagianni et al. 2008; Rottach et al. 2010). Although the phenotypes in *UHRF1* knockout or knockdown studies indicate that the localization of UHRF1 to replicating heterochromatin is dependent on DNA methylation (Bostick et al. 2007; Sharif et al. 2007), the ability of UHRF1 to bind methylated H3K9 may facilitate the recruitment of Dnmt1 to chromatin containing both H3K9 methylation and hemimethylated DNA, and, hence, Dnmt1-mediated full methylation.

Nonhistone substrates of G9a and GLP

As mentioned previously, most of the SET domain lysine methyltransferases have been described as HKMTs. However, the SET domain molecule SETD6 methylates RelA (K310), a subunit of NF-κB, but not histones (Levy et al. 2011). In addition to self-methylation, G9a and GLP also methylate nonhistone substrates, including p53 (K372), Wiz (K305), CDYL1 (K135), ACINUS (K654), and Reptin (K67) (Sampath et al. 2007; Rathert et al. 2008; Huang et al. 2010; Lee et al. 2010). Currently, the biological roles of these nonhistone methylations are not well understood.

How are target specificities of G9a and GLP regulated?

As described above, G9a and GLP are the main HKMTs for H3K9me1 and H3K9me2 in euchromatin. Genome-wide analysis of different histone modifications suggests that the H3K9me2 mark is linked with transcriptional repression (Barski et al. 2007). In comparison with other histone methyl marks, H3K9me2 shows much longer spatial and continuous distribution on megabase-long stretches of genomic DNA (Wen et al. 2009). Thus, these data suggest that G9a/GLP-mediated H3K9me2 is integral to the establishment of these facultative heterochromatin domains, which are contained within larger expanses of euchromatin. Furthermore, in mouse ES cells, most of the G9a-repressed genes that are marked with H3K9me2 by G9a and derepressed by G9a inactivation are localized to the nuclear periphery (Yokochi et al. 2009). In human cells, nuclear lamina-associated large chromatin domains are transcriptionally repressed and relatively highly methylated with H3K9me2 (Guelen et al. 2008). Whether this nuclear lamina association is important for target specificities of H3K9me2 deposited by the G9a–GLP complex or is simply linked with transcriptional silencing is not clear. In any case, the H3K9me2 mark is not essential for the nuclear peripheral localization of specific chromatin loci because the G9a-repressed genes remain in the nuclear periphery in ES cells after G9a inactivation and a reduction in H3K9me2 levels (Yokochi et al. 2009).

Many G9a-interacting molecules have been reported (Table 1). Most of these are characterized as repressive chromatin proteins and also multi-zinc finger molecules. Biochemically, G9a and GLP efficiently methylate histone octamers in vitro, but exhibit an extremely low rate of catalysis for nucleosomal histones (Tachibana et al. 2005; Shinkai 2007). Based on these molecular properties of G9a and GLP, we propose the following model for the G9a–GLP-mediated H3K9 methylation reaction in vivo. The G9a–GLP complex localizes to specific chromatin loci due to multiple interactions of the complex with chromatin or sequence-specific DNA-binding molecules. During replication, the G9a–GLP complex deposits K9me2 marks on newly synthesized H3 just before its incorporation into chromatin. Consistent with this notion, G9a colocalizes with replication foci during S phase (Estève et al. 2006).

H3K9me2 spreading and H3K9me binding by G9a and GLP ankyrin repeats (ANKs)

Because the chromodomain of HP1 binds to H3K9me2 and H3K9me3, and because H3K9 HKMT Suv39h1 itself interacts with HP1, a self-enforcing spreading mechanism has been proposed (Bannister et al. 2001; Lachner et al. 2001). The prevalence of H3K9me2 in megabase-long chromatin domains suggests that the contribution of the spreading mechanism is much greater for H3K9me2 than for H3K9me3. HP1 also exists in G9a- and GLP-containing complexes (Ogawa et al. 2002; Nishio and Walsh 2004; Nozawa et al. 2010), and self-methylation of G9a is involved in the G9a–HP1 interaction (Sampath et al. 2007). Furthermore, the ANKs of G9a and GLP also bind to H3K9me1 and H3K9me2 (Collins et al. 2008). Therefore, once the G9a–GLP complex initiates an H3K9 methylation reaction at a specific target locus, these multi-H3K9me-binding modules (HP1 chromodomain plus G9a and GLP ANKs) may enhance the spreading of H3K9me2 marks, again via G9a/GLP-mediated methylation reactions (Collins and Cheng 2010).

Development of G9a and GLP inhibitors

Lysine methyltransferases, including SET domain-containing molecules, transfer one to three methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino group of the target lysine residue. Chaetocin, a competitive inhibitor of SAM, was the first HKMT inhibitor to be discovered, and it specifically inhibits the enzymatic activities of HKMTs belonging to members of the SUV39 family, including SUV39H1, dSU(VAR)3-9, G9a, DIM-5, GLP, and ESET (Greiner et al. 2005). Another more specific H3K9 HKMT inhibitor, BIX01294 (BIX), has also been described (Kubicek et al. 2007). BIX was originally reported as a G9a-specific inhibitor (half maximal inhibitory concentration [IC_{50}] = 1.7 μM), but has been shown lately to exhibit a more potent inhibitory activity against GLP (G9a IC_{50} = 1.9 μM vs. GLP IC_{50} = 0.7 μM [Chang et al. 2009]; G9a IC_{50} = 250 nM vs. GLP IC_{50} = 27 nM, [Quinn et al. 2010]). More potent G9a/GLP inhibitors have been developed by further modification of BIX (E72: GLP IC_{50} = 100 nM [Chang et al. 2010]; UNC0321: G9a IC_{50} = 9.0 nM and GLP IC_{50} = 15 nM, [Liu et al. 2010]). Structural comparison of GLP bound to BIX with that of GLP bound to a substrate peptide suggests that the BIX family of small molecules acts as competitive

Table 1. *G9a-interacting molecules*

Gene name	Molecular function	Unique domain or region[a]	Reference for G9a interaction
CDP/Cut	Transcriptional repression, chromatin and DNA binding	Coiled coil, transmembrane, transmembrane helix	Nishio and Walsh 2004
E2F6	Transcriptional repression, DNA binding	Leucine zipper, DEF box	Ogawa et al. 2002
Gfi1/zfp163	Transcriptional repression, DNA binding, zinc ion binding	Zinc finger (C2H2-type), Ala/Gly-rich	Duan et al. 2005
Gfib	Transcriptional regulation, DNA binding, zinc ion binding	Zinc finger (C2H2-type)	Vassen et al. 2006
PRDM1/Blimp1	Transcriptional repression, DNA binding, zinc ion binding	Zinc finger (C2H2-type), PR	Gyory et al. 2004
NRSF/REST	Transcriptional repression, chromatin and DNA binding	Zinc finger (C2H2-type), Lys-rich, Pro-rich	Roopra et al. 2004
PRISM/PRDM6	Transcriptional repression, DNA binding, zinc ion binding	Zinc finger (C2H2-type), PR, Pro-rich	Davis et al. 2006
ZNF217	Transcriptional repression, DNA binding, zinc ion binding	Zinc finger (C2H2-type)	Banck et al. 2009
UHRF1/Np95	Transcriptional repression, DNA methylation, methyl-CpG binding, methylated histone binding	Ubiquitin-like, zinc finger (PHD-type and RING-type), methyl-CpG binding, YDG	Kim et al. 2009
HP1	Heterochromatin, transcriptional repression, chromatin binding, protein binding, methylated histone binding	Chromo, chromoshadow	Nozawa et al. 2010
DNMT1	DNA methylation, transcriptional repression, zinc ion binding, protein binding	BAH, zinc finger (CXXC-type), DNA methyltransferase region,	Estève et al. 2006
Dnmt3a, Dnmt3b	Transcriptional repression, DNA methylation, zinc ion binding, protein binding	Zinc finger (ADD-type), PWWP, DNA methyltransferase region	Epsztejn-Litman et al. 2008
Wiz	Zinc ion binding	Zinc finger (C2H2-type)	Ueda et al. 2006
ZNF200	Transcriptional regulation	Zinc finger (C2H2-type)	Nishida et al. 2007

[a]Underlined items are domains responsible for G9a interaction.

inhibitors of the N-terminal peptides of H3 (K4 to R8 or K9) (Chang et al. 2010). It is worth noting that the G9a/GLP inhibitor activity of BIX is robust if an H3 N-terminal oligopeptide is used as a substrate for the in vitro methyltransferase assay, but is not significant (no inhibition at 10 μM) if full-length H3 is used (Y Shinkai, unpubl.).

Roles of G9a and GLP: relevance to various biological processes in mammals and human diseases

Since G9a and GLP are expressed ubiquitously and function as major euchromatic H3K9me1 and H3K9me2 HKMTs, multiple biological roles have been proposed (Fig. 2). Both G9a and GLP play an important role in mouse development, as shown by the fact that *G9a* and *GLP* knockout lead to embryonic lethality around embryonic day 9.5 (E9.5) due to severe growth defects (Tachibana et al. 2002, 2005). Importantly, mutant mice with catalytically inactive G9a HKMT also have an embryonic lethal phenotype similar to that of *G9a* knockout mice (M Tachibana and Y Shinkai, unpubl.), suggesting that G9a-mediated H3K9 methylation is important for mouse development. Furthermore, G9a may regulate the expression of some imprinted genes (Nagano et al. 2008;

Wagschal et al. 2008). Germ lineage-specific *G9a* knockout also induces developmental defects of germ cells, and completion of meiosis is not observed in either sex (Tachibana et al. 2007). In the case of lymphocyte-specific *G9a* knockout, lymphocyte development is mostly intact; however, some immune responses are affected or impaired. B-cell-specific *G9a* knockout mice have a reduced usage of Igλ L chains, and a reduction in Igλ gene assembly in bone marrow precursor cells is also seen. *G9a*-deficient B cells do not respond well to LPS + IL-4 in
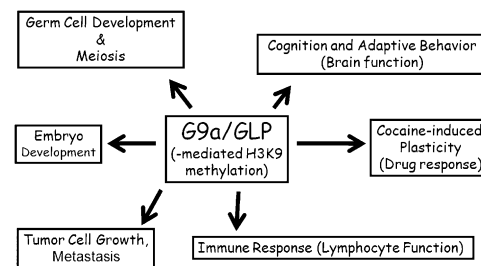

Figure 2. Multiple biological roles of G9a/GLP. Multiple biological roles of G9a/GLP are proposed, based mainly on the results of studies using *G9a* or *GLP* knockout mice.

vitro (Thomas et al. 2008). T-cell development in the thymus is intact in T-cell-specific *G9a* knockout mice; however the expression of lineage-specific Th2-associated cytokine genes such as *IL-4*, *IL-5*, and *IL-13* are impaired, and the mice fail to defend themselves against parasitic infections (Lehnertz et al. 2010).

Phenotypes of the postnatal and forebrain neuron-specific *G9a* or *GLP* knockout mice are interesting (Shaefer et al. 2009). In such mice, multiple nonadult neuronal genes are derepressed in the forebrain, but there are no obvious neuronal developmental or architectural defects. However, these mice show various abnormal phenotypes, especially defects in cognition and adaptive behaviors. Similar roles of G9a in neuronal function are conserved in *Drosophila*, and *EHMT/G9a*-deficient flies show defects in learning and memory (Kramer et al. 2011). One copy of the *EHMT1/GLP* gene is deleted or disrupted in 9q34.3 subtelomeric deletion syndrome patients with severe mental retardation (Kleefstra et al. 2005, 2006). Indeed, not only forebrain-specific *GLP* homozygous knockout mice but also the heterozygous knockout mice show abnormal behaviors, including reduced locomotor activity and exploration (Shaefer et al. 2009). In addition, complete *GLP* knockout heterozygous mice also show reduced exploration, increased anxiety, and altered social behavior (Balemans et al. 2010). These results suggest that reduced function of G9a, GLP, or the G9a–GLP complex in the forebrain may, in part, lead to the phenotypes of 9q34.3 subtelomeric deletion syndrome patients.

Interesting findings have further been reported regarding a critical role of G9a-mediated H3K9me2 in cocaine-induced neuronal responses (Maze et al. 2010). Repeated cocaine exposure induces alterations in gene expression in the nucleus accumbens (NAc) neurons in mice, and this change is critical for cocaine-induced changes in neural morphology and behavior that may underlie cocaine addiction. Of the known H3K9 and H3K27 HKMT and HKDM genes, the expression levels of *G9a* and *GLP* are specifically diminished in the NAc neurons by repeated cocaine exposure in mice. Consistent with this finding, levels of H3K9me2 are also significantly reduced. If this cocaine-induced *G9a* reduction is complemented by exogenous *G9a* expression, the cocaine-induced neuronal morphological and behavioral changes are suppressed. On the other hand, if G9a function is inactivated specifically in NAc neurons, the neuronal morphological changes are enhanced even without cocaine exposure, and the behavioral preference for cocaine is further enhanced. Repeated cocaine exposure induces the accumulation of the transcription factor ΔFosB in NAc neurons. ΔFosB represses *G9a* expression, and the consequent reduction of H3K9me2 induces up-regulation of G9a-repressed genes. If these molecular mechanisms in mice can be extrapolated to cocaine addiction in humans, G9a (G9a–GLP complex) or the HKMT activity of G9a would be a novel target for controlling cocaine addiction.

In addition, *G9a* and *GLP* have also both been found to be up-regulated in various types of human cancer cells (Huang et al. 2010). Consistent with this finding, knock-down of *G9a* suppresses tumor cell growth in vitro and the invasion of cancer cells in nude mice (Kondo et al. 2008; Chen et al. 2010).

Finally, G9a is involved in provirus silencing. A provirus is a viral genome that is integrated into the host genome, and it is generally in the cell's best interest to silence proviruses. Such silencing in mouse ES cells is effected by another H3K9 HKMT, ESET/SETDB1/KMT1E (Matsui et al. 2010). G9a and GLP control H3K9me2 and DNA methylation of endogenous retroviruses (ERVs) in mouse ES cells (Dong et al. 2008). However, ERVs are not reactivated in *G9a* or *GLP* knockout ES cells, since ESET and ESET-mediated H3K9me3 are still maintained on the ERVs (Dong et al. 2008; Matsui et al. 2010). Further characterization indicates that newly integrated active proviruses are not silenced efficiently in *G9a* knockout ES cells, suggesting that G9a is dispensable for maintenance but is critical for establishment of proviral silencing in ES cells (Leung et al. 2011).

Concluding remarks

H3K9 methylation is a highly conserved histone post-translational modification and is commonly linked with both facultative and constitutive heterochromatin formation and transcriptional repression. Furthermore, the proteins that bind to methylated H3K9 (HP1/Swi6) are also conserved. Studies using *G9a* or *GLP* knockout/knockdown mice and cells also reported that G9a/GLP-mediated H3K9me2 is highly linked with transcriptional silencing. However, it is still unclear whether HP1 recruitment and its downstream signaling pathway are the primary mechanisms for H3K9me2- or H3K9me3-mediated gene silencing, or whether other unknown mechanisms are more important. Recently, a subset of G9a/GLP has been shown to form a complex with Suv39h1 and ESET (Fritsch et al. 2010). As discussed for the G9a/GLP complex, it is interesting to address the functional significance of a complex possessing multiple (four in this case) HKMTs with the same substrate (H3K9) specificity. Finally, in plants and the yeast *Schizosaccharomyces pombe*, the RNAi machinery is integrated with the regulation of H3K9 methylation (Grewal 2010; Tamaru 2010). While the RNAi machinery is also conserved in mammals, it is not clear whether this system is involved in G9a/GLP-mediated H3K9 methylation.

Dysregulation of histone methylation may have a role in various human diseases. Recent reports strongly support this notion, and it is likely that more evidence will be reported in the future. It is obvious that efficient animal cloning by somatic nuclear transfer and the establishment of induced pluripotent stem (iPS) cells depends on the efficiency of epigenetic reprogramming. If dysregulation of histone modification is the underlying cause of some human diseases and this epigenetic dysregulation can be reprogrammed, then it may be possible for such diseases or abnormalities to be reset or cured. From this point of view, the abnormal behavioral phenotypes of the forebrain-specific *G9a/GLP* knockout mice (Schaefer et al. 2009) and the obese phenotype of the *H3K9 HKDM*

Jmjd1a/JHDM2A/KDM3a knockout mice (Inagaki et al. 2009; Tateishi et al. 2009) are quite interesting. If genetic or pharmacological approaches can improve these knockout mice after the onset of phenotypes, then an epigenetic reprogramming strategy may be applicable to a variety of biological aims.

Acknowledgments

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H3K9 methyltransferase G9a and the related molecule GLP

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