Mechanisms of epigenetic inheritance
Cyrus Martin and Yi Zhang

The mechanisms by which stable gene expression patterns are inherited during cell division are not well understood. Chromatin is subject to a number of covalent modifications and it is generally believed that the transfer of these modifications between cell generations plays a critical role in inheritance, though how this occurs is a matter of debate. In one proposed model, replication of chromatin in a semi-conservative fashion would allow 'template reading' and 'writing' mechanisms to copy modifications from old histones to new histones. Conversely, if chromatin is replicated in a conservative fashion, then other mechanisms, such as the replacement and/or modification of histones during transcription, may mediate the replication of these modifications. Finally, several recent studies suggest that the faithful replication of DNA methylation patterns may be used to propagate histone modifications associated with gene silencing.

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Introduction

The word 'epigenetics' refers to the study of inherited phenotypic variations that are not the result of variations in DNA sequence. This term encompasses a number of phenomena, including genomic imprinting, paramutation, Polycomb silencing and position effect variegation. Paramutation, for example, which was first described in plants, is a phenomenon in which one allele of a gene specifying a trait, such as seed color, alters or 'paramutates' the homologous allele [1]. Importantly, the paramutated allele and associated phenotype can be passed to subsequent generations, which is called transgenerational epigenetic inheritance [2]. Unlike classically inherited traits, which are determined by DNA sequence, the phenotypes observed in paramutation phenomena are the result of stable changes in the expression of the affected alleles. Recent studies of paramutation in mice indicate that small RNA

molecules may mediate the transmission of paramutated alleles between generations, although the mechanism by which this occurs remains uncertain [3•].

The term epigenetics has also been applied to the stable propagation of gene expression programs during cell division, referred to as mitotic epigenetic inheritance [2]. Observations made in studies of *Drosophila* development illustrate this concept well. It has been demonstrated, for example, that embryonic cells responsible for the formation of adult structures in the fly can go through many cell divisions when removed from their native environment and yet still retain the ability to form the appropriate structure, for example wing or leg, when reintroduced back into the developing embryo [4]. This suggests that these cells not only 'remember' their identity, but also pass on this information during cell division. How is this achieved? It is currently believed that covalent modification of both DNA and histones, the proteins responsible for packaging DNA, underlies these epigenetic phenomena. Thus these modifications appear to dictate specific gene expression patterns and, in some instances, may be replicated and passed on to daughter cells.

DNA and histones are assembled into nucleosomes, the fundamental building blocks of chromatin. Specifically, the nucleosome consists of 147 base-pairs of DNA wrapped around an octamer of four core histone proteins: H2A, H2B, H3 and H4. Work over the past 15 years has led to a comprehensive understanding of the nature of covalent modifications to DNA and histones, the enzymes responsible and, in some cases, the link between particular modifications and gene expression. Covalent modification of DNA in eukaryotes is restricted to methylation of cytosine bases and this modification is associated with gene silencing [5]. Histone proteins, in contrast to DNA, are subject to a large number of modifications that include methylation, acetylation, ubiquitylation and phosphorylation. These modifications are associated with both gene silencing and activation, depending on the nature of the modification and the specific amino acid modified [6–9]. Although some histone modifications may influence gene expression by affecting chromatin folding, the current paradigm is that most modifications influence interactions between factors that regulate gene expression and chromatin. Assuming that chromatin modifications mediate epigenetic phenomena, a key question that remains is the precise mechanism by which these modifications are propagated during cell division. In the case of DNA methylation, the problem appears to be solved. The replication of methylated DNA results in

Figure 1

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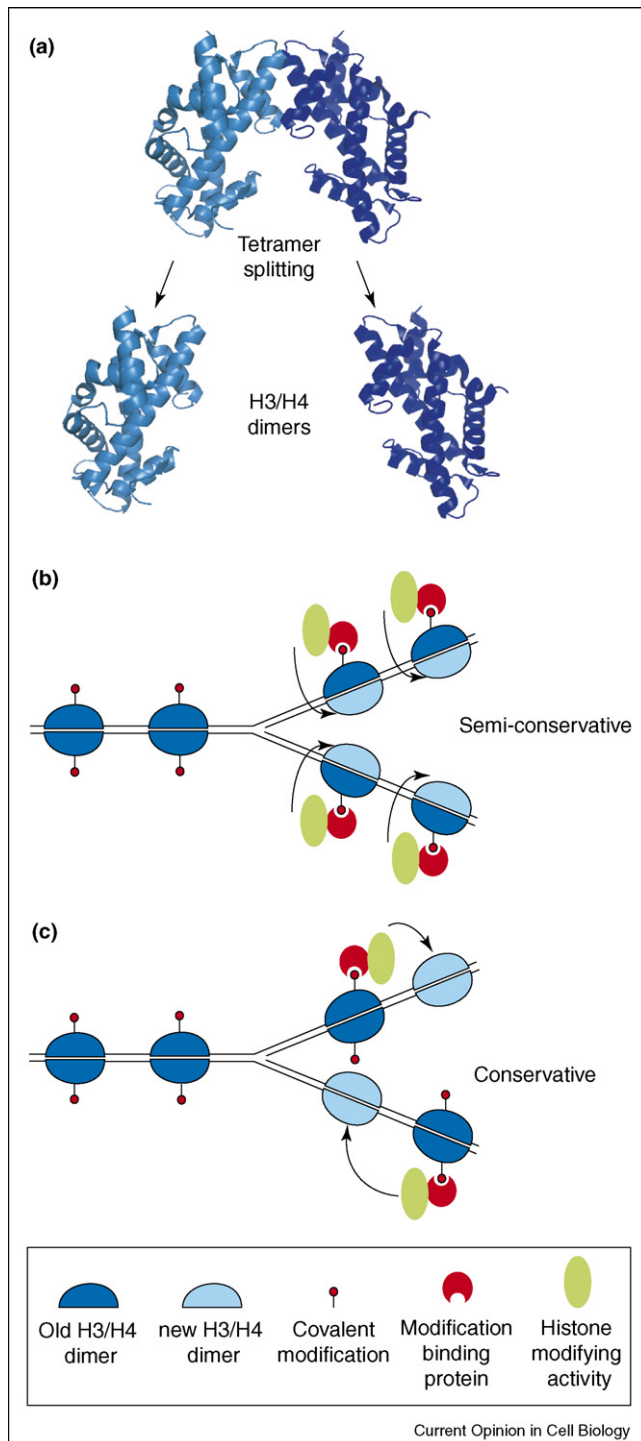
Replication-dependent chromatin assembly and implications for the inheritance of histone modifications. **(a)** Histone H3 and H4 form a tetramer as a result of protein–protein interactions between two H3/H4 dimers. Under special circumstances, it is possible that the tetramer may be split during the replication of chromatin. **(b)** In the first model of chromatin assembly during DNA replication, the H3/H4 tetramer is split such that nucleosomes on daughter strands are assembled with one old H3/H4 dimer and one new H3/H4 dimer. In this schematic presentation, the histone modifications on the old

hemi-methylated sites, which are the preferential substrate of DNMT1, a DNA methyltranserase that is associated with the replication machinery [5,10]. Much less is known regarding the replication and inheritance of histone modifications, although a handful of studies in the past few years hint at potential mechanisms. Here we discuss the implications of those findings for models of epigenetic inheritance.

Chromatin assembly pathways and epigenetic inheritance

The replication of chromatin involves both DNA synthesis and nucleosome assembly, two processes that occur coordinately during S-phase of the cell cycle. Assembly of nucleosomes during DNA synthesis on the two daughter strands involves both the redistribution of histones that were previously associated with the parental DNA strand and the deposition of newly synthesized histones, referred to as replication-coupled (RC) nucleosome assembly. Considering histones H3 and H4 alone, an examination of the nucleosome structure shows that the tetramer formed by H3 and H4 could theoretically be split into two identical H3/H4 dimers (Figure 1a). In the context of RC chromatin assembly, tetramer splitting might allow an old H3/H4 dimer to be reassembled with a newly synthesized H3/H4 dimer such that each daughter DNA strand receives a hybrid tetramer [11••]. This model, referred to as semi-conservative chromatin assembly, is consistent with the fact that the pre-deposition form of H3/H4 appears to be a dimer and not a tetramer [12]. In addition, it has been recently demonstrated that certain chaperone proteins required for RC chromatin assembly interact specifically with H3/H4 dimers and can split H3/H4 tetramers *in vitro* [13].

If the semi-conservative chromatin assembly model is correct, it provides a potential solution to the problem of inherited histone modifications (Figure 1b). Specifically, the epigenetic information contained within each old H3/H4 dimer could be used as a 'template' to copy information onto the new H3/H4 dimer, thus fully replicating the original nucleosome and its associated modifications. Interestingly, examples of potential template 'reading' and 'writing' mechanisms have already been demonstrated. For example, the methylation of H3 lysine 4 and lysine 9 leads to the recruitment of the methyl-binding proteins WDR5 and HP-1, respectively [14•].

(Figure 1 Legend continued) dimer are recognized by specific binding domains, leading to the recruitment of enzymatic activities that copy histone modifications onto the new H3/H4 dimer. **(c)** In the second model, old tetramers are distributed intact to the daughter strands such that nascent chromatin contains a mix of nucleosomes assembled with old tetramers and nucleosomes assembled with newly synthesized tetramers. As in the first model, histone modifications on old histones would recruit enzymatic activities, but in this case those activities would be directed towards newly synthesized histones in adjacent nucleosomes.

Importantly, both proteins directly interact with enzymes that catalyze the methylation of lysine 4 and 9, suggesting that these modifications could potentially recruit enzymatic activities to an adjacent, unmodified H3 molecule in a hybrid nucleosome. To further validate the template reading and writing mechanism suggested by these observations it will be necessary to directly analyze the interplay between modifications on adjacent H3 molecules in the same nucleosome. Such experiments await the development of tools capable of studying individual molecules.

The notion of semi-conservative nucleosome replication is by no means new and has been tested by a number of methodologies, most convincingly by pulse-chase experiments with isotopic labels (reviewed in [15,16]). Most studies examining bulk chromatin have concluded that the H3/H4 tetramer remains intact during DNA replication, arguing against semi-conservative replication (Figure 1c). Interestingly, a single study focusing on genomic regions associated with gene activity provided evidence for semi-conservative replication, suggesting that this model may apply in special situations [17]. It is known that actively transcribed regions have a unique nucleosome architecture. Of particular relevance is the fact that the form of H3 deposited during DNA replication, termed H3.1, is replaced with an H3 variant termed H3.3 during active gene transcription in a replication-independent (RI) chromatin assembly pathway [18]. Perhaps H3.3 or an associated modification instructs the chromatin assembly machinery to split the nucleosome in half during DNA replication. A prediction that this refined model makes is that the replication of H3.3-containing nucleosomes should result in the formation of hybrid nucleosomes containing one molecule each of H3.1 and H3.3. Arguing against this model is the fact that mononucleomes isolated from bulk chromatin are homogenous, containing either H3.1 or H3.3; however, this could be reconciled with semi-conservative replication if H3.1 were rapidly replaced in the hybrid nucleosome by H3.3 during gene transcription [11••]. Considering the potential complications posed by the transcription process itself, it may be useful to examine the replication of H3.3-containing chromatin *in vitro* using DNA-synthesis-dependent chromatin assembly assays.

Alternatives to tetramer splitting and semi-conservative nucleosome replication have been proposed to explain epigenetic inheritance [16]. For example, it is known that in bulk chromatin old histone octamers segregate randomly to both daughter strands during DNA replication, with newly synthesized octamers filling in the gaps [19]. It is possible that the template 'reading' and 'writing' mechanism discussed above also applies in this situation, such that the modifications present in an old nucleosome would stimulate similar modifications in adjacent, newly assembled nucleosomes (Figure 1c). Evidence supporting

this concept has emerged from studies of heterochromatin formation in fission yeast. In this system, it has been observed that domains of H3K9 methylation associated with heterochromatin shrink in the absence of the methyl-H3K9 'reader' Swi6 [20]. This result suggests a reiterative process whereby Swi6 binds to a methylated nucleosome and recruits methyltransferase activity to adjacent nucleosomes, resulting in the creation of new Swi6 binding sites and the outward expansion of H3K9 methylation. With regard to alternative mechanisms of epigenetic inheritance at actively transcribed genomic regions, it has been posited that covalent modifications associated with old nucleosomes might stimulate a low level of transcription, leading to the replacement and/or modification of histones (Figure 2) [16]. In one scenario, newly synthesized H3.3 would be covalently modified prior to deposition and the transcription process would lead to the replacement of nucleosomes by RI chromatin assembly. Interestingly, the pre-deposition form of H3.3 has been demonstrated to be covalently modified [21•]. In the second scenario, enzymatic activities associated with the transcription process itself would lead to the covalent modification of H3.1 or newly deposited H3.3. This mechanism is strongly supported by a number of studies demonstrating the association of the elongating form of RNA polymerase II with histone modifying activities (reviewed in [7]).

Histone methylation directed by DNA methylation — the 'piggy-back' model

Given that mechanisms exist to faithfully replicate DNA methylation patterns, it is tempting to speculate that these mechanisms might be exploited to replicate histone modifications as well. In one possible model, methylated DNA would direct histone modifying activities to specific target genes, resulting in gene silencing (Figure 3). If it is assumed that the DNA methylation pattern is replicated accurately by DNMT1 during DNA synthesis, the process would theoretically be repeated on nascent chromatin, leading to the replication of epigenetic information. In this model, the histone modifications could be viewed as 'piggy-backing' on the replicated DNA methylation pattern.

There are several studies that support the recruitment aspect of this model. For example, it has been demonstrated that the methyl-CpG binding protein MeCP2 recruits the H3K9 methyltransferase SUV39H1 to target genes, leading to H3K9 methylation and gene silencing [22]. In addition, two independent studies have linked histone methylation to both DNA methylation and replication. The earlier of the two studies demonstrated that DNA methylation is necessary to recruit a complex containing the H3K9 methyltransferase SETDB1 and MBD1, a protein that interacts specifically with methyl-CpG [23••]. In this particular case SETDB1 appears to target DNA replication forks and, interestingly, histone methylation requires progression through

Figure 2
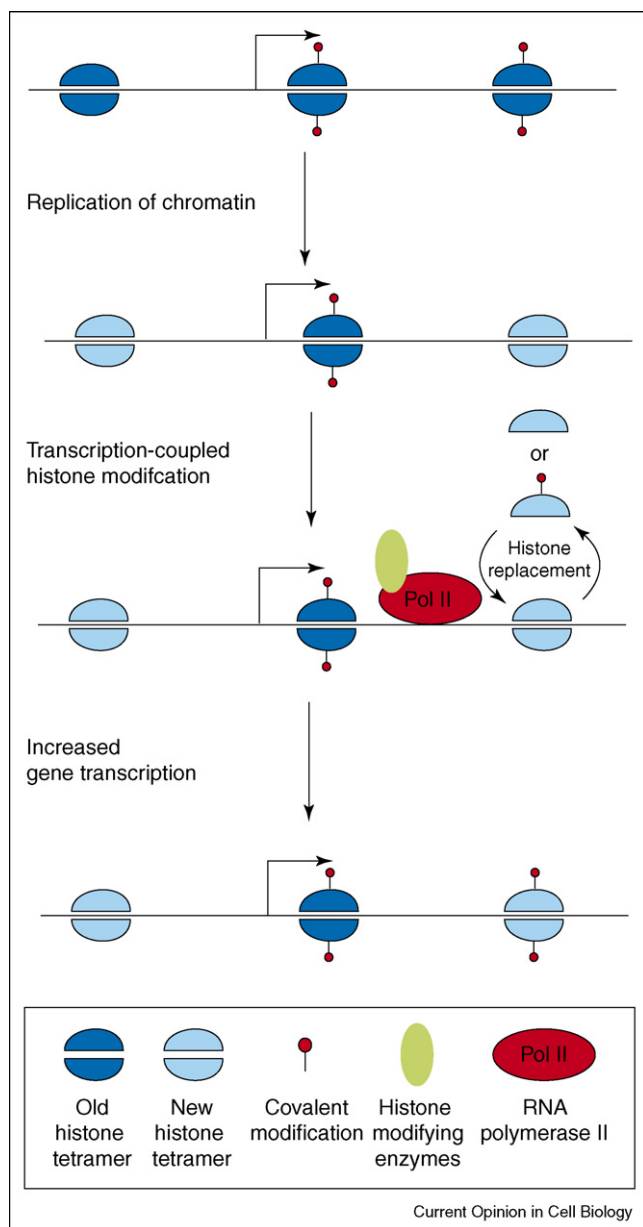
Transcription-coupled inheritance of histone methylation. In the model illustrated here, an actively transcribed gene is replicated during S-phase such that the nascent chromatin consists of nucleosomes assembled with old H3/H4 tetramers interspersed with nucleosomes assembled with new, unmodified H3/H4 tetramers (conservative model). The old nucleosomes, owing to specific covalent modifications, stimulate a low level of transcription that has the effect of resetting the original epigenetic information. In one possible scenario, the chromatin assembly activity associated with gene transcription (RI chromatin assembly) would lead to the deposition of covalently modified H3.3. In the second scenario, newly synthesized histones deposited during S-phase or H3.3 molecules deposited during transcription would be modified by enzymatic activities associated with RNA polymerase. Importantly, these events do not have to be mutually exclusive.

S-phase. The second study examined the connection between G9a, another H3K9 methyltransferase, and DNA methylation [24••]. Like SETDB1, G9a was also found to be associated with replication forks and this interaction was dependent on DNMT1. In contrast to the mechanism of recruitment of SETDB1, which is mediated by a methyl-CpG-binding protein, DNMT1 can directly recruit G9a to the replication fork through protein–protein interactions. This observation raises the possibility that G9a-mediated histone methylation may not be strictly dependent on DNA methylation *per se*, a property that might allow the enzyme to methylate histones in genomic regions that are devoid of DNA methylation under certain circumstances.

The association of both SETDB1 and G9a with the replication fork is significant because it suggests that these histone methyltransferases function to methylate newly synthesized and assembled histones, a finding that is consistent with a potential role for these enzymes in the replication of histone methylation. Questions still remain, however, regarding how DNA methylation might be transduced to replicate histone modifications. For example, why is progression through S-phase required for SETDB1-mediated histone methylation? One possibility is that the methylated DNA sequence recognized by MBD1 is masked when assembled into nucleosomes, but becomes accessible when the replication fork passes through this sequence as a result of nucleosome disruption. With regard to G9a, the relationship between DNA methylation and the establishment of histone methylation remains unclear. Unlike SETDB1, proteins that recognize methylated DNA have not been demonstrated to interact with G9a or mediate its recruitment, although this remains a possibility. An alternative mechanism could involve the coupling of G9a methyltransferase activity with DNMT1 catalytic activity. Interestingly, the interaction of DNMT1 has been demonstrated to modestly stimulate G9 methyltransferase activity *in vitro* [24••].

It is important to point out that the piggy-back model cannot explain the inheritance of all histone methylation associated with gene silencing. This can be easily deduced from the fact that some organisms lack DNA methylation, yet employ histone methylation for the purposes of gene silencing. Another issue of relevance to the validity of the model is the fact that many studies have demonstrated that DNA methylation acts downstream of histone methylation [25–27]. Most recently, for example, it was shown that the H3K27 methyltransferase EZH2 interacts with DNA methyltransferases and directs DNA methylation to targets, a function which appears necessary for gene silencing [28•]. In this case, it would appear that DNA methylation is dependent on histone methylation, the converse of what the piggy-back model would predict. It is important to note, however, that in

Figure 3
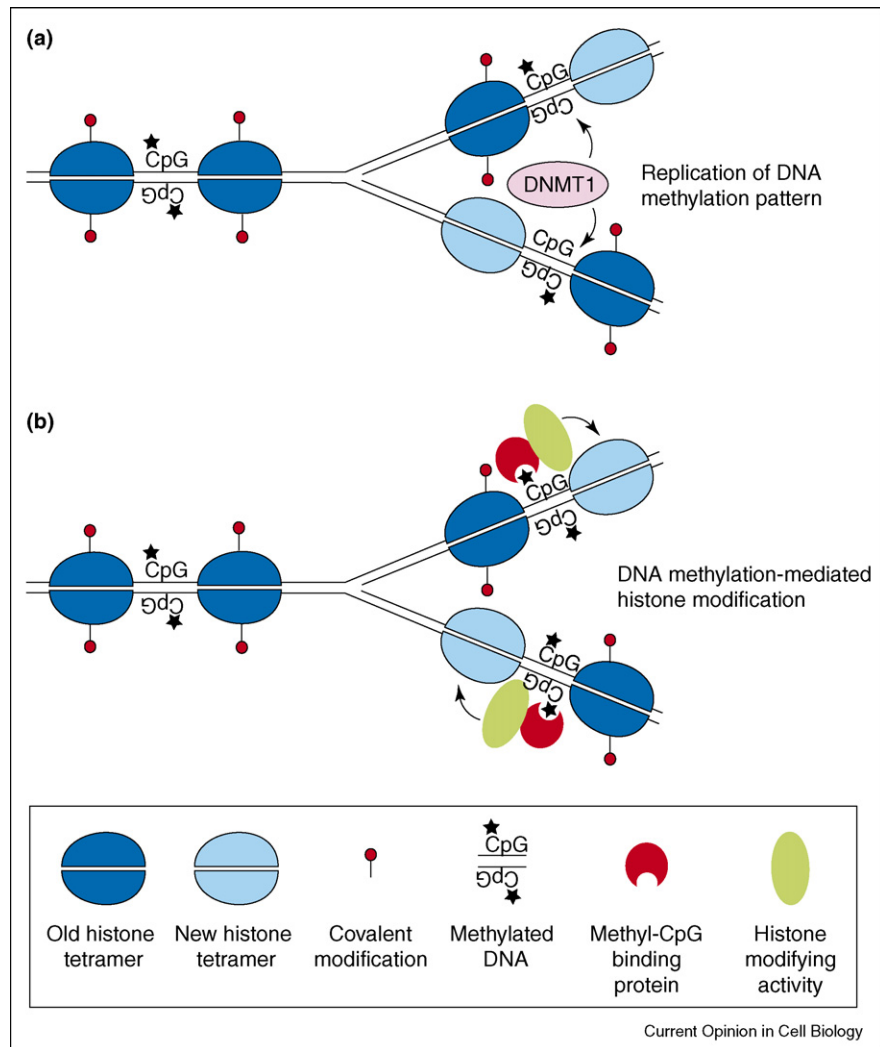
DNA methylation and the 'piggy-back' mechanism. **(a)** It is known that the DNA methyltransferase DNMT1 associates with the DNA replication machinery and plays a critical role in the replication of DNA methylation patterns. Current evidence suggests that DNMT1 specifically targets hemi-methylated sites in newly synthesized DNA, resulting in the faithful replication of DNA methylation patterns. **(b)** It is possible that the accuracy associated with copying DNA methylation patterns is utilized by the cell to replicate histone modifications as well. In one potential mechanism shown here, methylated DNA, through the recruitment of a methyl-CpG binding protein, would act to recruit histone modifying activities after each round of DNA replication, such that the establishment of histone modifications would 'piggy-back' on DNA methylation.

this and other studies the dependence of histone methylation on DNA methylation was not examined. One important exception was a study of heterochromatin formation in plants, which found that H3K9 methylation did not depend on DNA methylation in all cases and that the loss of this modification may simply reflect transcriptional activity [29]. These examples indicate that the piggy-back model may not always apply.

Alternatively, the basic tenets of the model may still hold but need to be elaborated to reflect a bi-directional relationship between DNA methylation and histone methylation. Suppose, for example, that there is an initiating event, such as the binding of a repressor, that leads to the recruitment of histone-modifying activities coincident with gene silencing. These enzymatic activities might then directly recruit DNA methyltransferases to loci, resulting in *de novo* DNA methylation. In this situation, the establishment of DNA methylation would depend on histone modifications. While the factors responsible for initiating silencing might be absent in subsequent cell generations, the piggy-back model as originally outlined would allow stable silencing through the faithful replication of DNA methylation and recruitment of histone modifying activities.

Conclusions

Our discussion has focused on potential mechanisms of epigenetic inheritance, particularly on the inheritance of histone methylation. We first presented several models that explain how modifications on old histones may direct the modification of newly synthesized histones to replicate patterns of histone methylation in nascent chromatin. Available evidence suggests that the propagation of histone methylation patterns may occur directly through 'template reading' and 'writing' mechanisms or indirectly as a result of gene transcription. In the second part of our discussion, we summarized recent evidence indicating that the inheritance of histone methylation associated with gene silencing may rely on the replication of DNA methylation patterns. Although there is some preliminary evidence to support such a mechanism, its generality has yet to be demonstrated. To this end, it will be important to comprehensively examine the role of DNA methylation in establishing various histone modifications associated with gene silencing. It will also be interesting to investigate the mechanistic details that underlie the coupling of DNA methylation and histone methylation during replication. In the past, *in vitro* assays have been successfully used to study chromatin assembly during DNA replication, but a DNA synthesis-dependent chromatin assembly system that incorporates DNMT1 has yet to be established. The development of such a system would be an invaluable tool to study mechanisms of epigenetic inheritance.

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