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Review

Histone lysine methylation and chromatin replication ☆

Carlos Rivera [a,1], Zachary A. Gurard-Levin [b,c,d,e,f,1], Geneviève Almouzni [b,c,d,e,f,*], Alejandra Loyola [a,**]

[a] Fundación Ciencia & Vida, Santiago, Chile
[b] Institut Curie, Centre de Recherche, Paris F-75248, France
[c] CNRS, UMR 3664, Paris F-75248, France
[d] Equipe Labellisée Ligue contre le Cancer, UMR 3664, Paris F-75248, France
[e] UPMC, UMR 3664, Paris F-75248, France
[f] Paris Sciences & Lettres, PSL, France

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ABSTRACT

In eukaryotic organisms, the replication of the DNA sequence and its organization into chromatin are critical to maintain genome integrity. Chromatin components, such as histone variants and histone post-translational modifications, along with the higher-order chromatin structure, impact several DNA metabolic processes, including replication, transcription, and repair. In this review we focus on lysine methylation and the relationships between this histone mark and chromatin replication. We first describe studies implicating lysine methylation in regulating early steps in the replication process. We then discuss chromatin reassembly following replication fork passage, where the incorporation of a combination of newly synthesized histones and parental histones can impact the inheritance of lysine methylation marks on the daughter strands. Finally, we elaborate on how the inheritance of lysine methylation can impact maintenance of the chromatin landscape, using heterochromatin as a model chromatin domain, and we discuss the potential mechanisms involved in this process. This article is part of a Special Issue entitled: Methylation: A Multifaceted Modification — looking at transcription and beyond.

1. Introduction

In eukaryotes, the genetic material is organized in a nucleoprotein-complex called chromatin. Within its building block, the nucleosome, 147 base pairs of DNA are wrapped around an octamer of histone proteins, including two copies each of the core histones H3, H4, H2A, and H2B [1]. This basic unit is versatile showing distinct variations including DNA methylation, histone variants, and post-translational modifications of histones (reviewed in [2,3]). In turn, these marks can either alter the structure directly or through recruitment of chromatin-binding proteins that impact the chromatin state and various processes acting on DNA including replication, transcription, and repair. Notably, to access to the DNA sequence during these processes chromatin organization is transiently disrupted. These dynamics can challenge the maintenance of information conveyed at a chromatin level and in turn could impact gene expression profiles, cell identity and function. Thus, in all of these instances a proper chromatin reassembly is a critical step to consider. DNA replication with a doubling of the genomic material poses a particular challenge since it is accompanied by a genome-wide effect on chromatin that undergoes destabilization and re-assembly on the two daughter strands. Therefore, replication has been envisioned as a window of opportunity to change the chromatin landscape and thus of importance to evaluate maintenance versus switch in gene expression profiles. In addition, an unfaithful duplication of the DNA sequence and its organization into chromatin could also affect genome function. Thus, coordinating the faithful duplication of the DNA sequence and its organization into chromatin is important to consider to maintain/alter genome and epigenome integrity.

Histone modifications have been implicated in regulating cellular activities at defined genomic loci (reviewed in [3,4]). In this review, we focus specifically on methylation of lysine residues. An extensively-documented correlation between methylation and a DNA metabolic process is transcription, where the enrichment in H3K9me3, H3K27me3, and H4K20me3 associates with transcriptionally inactive regions, contrasting with H3K4me3 and H3K36me3 that mark transcriptionally active regions [3,4]. The recent emergence of a connection between histone lysine methylation and DNA replication lead us to review these pioneering studies in order to address how methylation at specific residues and to different extents (either mono-, di-, or trimethylation) impact early steps in DNA replication. In turn, we discuss how DNA replication impacts the propagation of these methylation marks to the daughter strands, an important parameter in epigenetic inheritance during

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* Correspondence to: G. Almouzni, CNRS UMR3664, Institut Curie, 26 rue d'Ulm Paris, 75248 France. Tel.: +33 1 56246706.
** Correspondence to: A. Loyola, Fundación Ciencia & Vida, Avenida Zañartu 1482, Ñuñoa, Santiago, Chile. Tel.: +56 2 23672048.
E-mail addresses: Genevieve.Almouzni@curie.fr (G. Almouzni), aloyola@cienciavida.cl (A. Loyola).

[1] These authors contributed equally to this work.

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cellular divisions. Finally, beyond the nucleosomal level, we review possible mechanisms that participate to restore the histone lysine methylation state on defined chromatin domains. For this we use pericentric heterochromatin as a model, a well-characterized chromatin domain enriched in H3K9me3 and H4K20me3, marks that are critical for proper centromere function and chromosome segregation.

2. Histone lysine methylation: a role in early replication steps?

We distinguish here four typical phases (Fig. 1) during replication in which we can highlight proposed roles for histone lysine methylation. First, recognition of replication origins by the origin recognition complex (ORC); second, recruitment during early G1 phase of the cell division control protein 6 (CDC6) and the minichromosome maintenance (MCM) complex to ORC binding sites, forming the pre-replication complex (preRC) in a process referred to as "licensing"; third, "firing" of the origins at the entry to S-phase, mediated by the action of kinases including the cell division control protein 45 (CDC45) (reviewed in [5–7]); fourth, "elongation" (reviewed in [6]). In this section we restrict our discussion to lysine methylation and how this mark on histones H3 and H4 may impact different aspects of DNA replication (Table 1).

2.1. Origin recognition

How origins are identified in metazoans remains largely unknown, and to date no consensus sequence has been described to sufficiently predict origin identification [8]. Thus, dynamic features determined by either DNA structural elements such as G-quadruplexes [9] or chromatin-related factors have attracted attention as they may provide a signal for early steps in DNA replication (for review see [10,11]). Here we highlight possible links between histone methylation and chromatin replication (Fig. 1). In a first example, peptide arrays of 82 histone peptides featuring different methylation marks enabled the identification of ORC components that bind to distinctly methylated peptides [12]. Human ORC1, a protein that features a bromo-adjacent homology (BAH) domain (reviewed in [13]), specifically binds a peptide presenting H4K20me2 [14]. Sub-cellular fractionation further supported this binding since abrogation of the BAH recognition domain impaired ORC1 binding

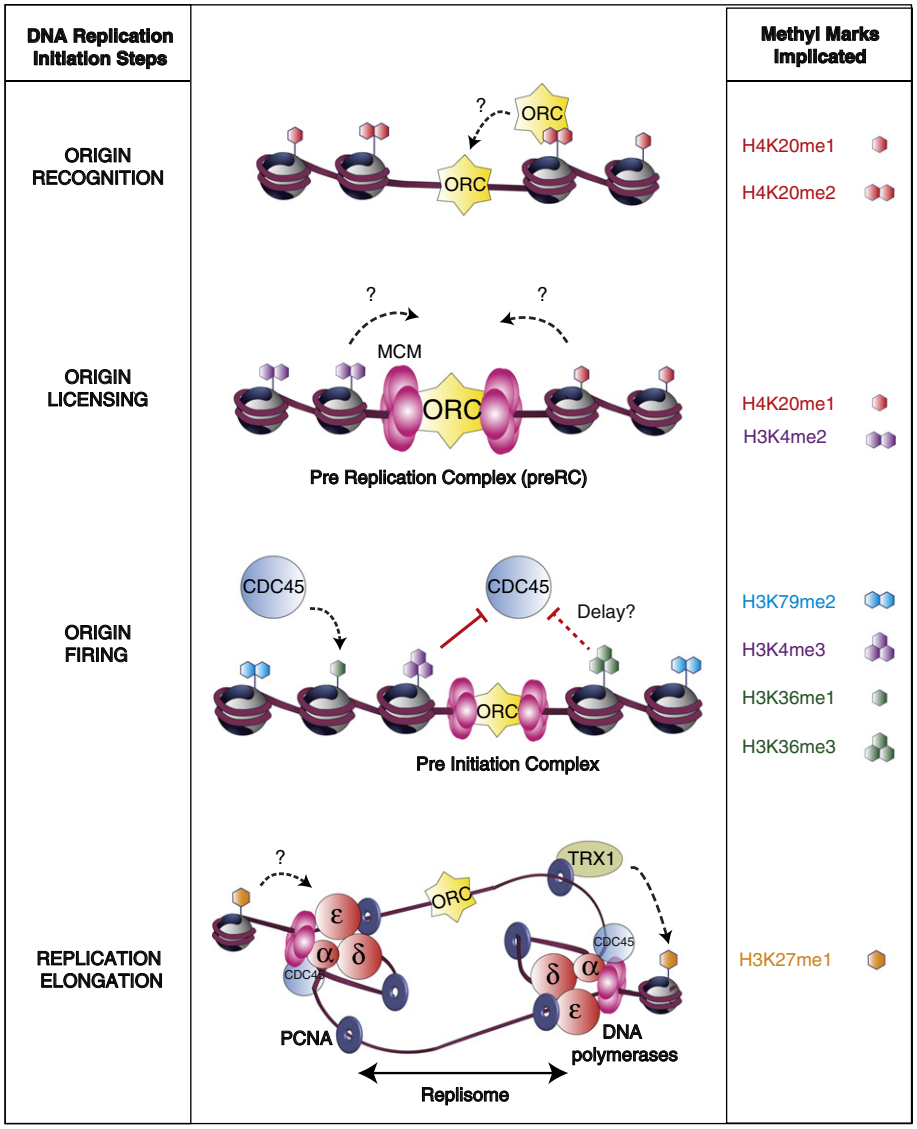

Fig. 1. Histone lysine methylation implicated in four phases of eukaryotic DNA replication. During the origin recognition step, H4K20me2 recruits ORC subunits to replication origins. For origin licensing, H4K20me1 is present at this stage, however, its role in promoting the assembly of the preRC remains unknown. In addition, H3K4me2 in yeast seems to be involved in this step. During origin firing, different methylation marks cooperate to regulate CDC45 binding to the pre-initiating complex. H3K36me1 likely recruits CDC45 whereas H3K36me3 may delay CDC45 loading, as this mark is found at late-replicating genes. H3K4me3 inhibits CDC45 binding. H3K79me2 is enriched during this phase, however its role remains unknown. Finally, TRX1 monomethylates H3K27 during elongation, however, the function of this mark remains elusive.

Table 1
Summary of the H3 and H4 lysine methylation implicated in the different DNA replication steps. The table describes their proposed methylase(s) and demethylase(s), the enrichment at genomic positions related to replication, and their roles in origin licensing, origin firing, replication elongation, and heterochromatin assembly.

Modification			Replication sites enrichment	Role in DNA replication steps	Methylase(s)	Demethylase(s)
Histone	Lysine	Degree				
H3	4	me1	Early origins	Not reported.	MLLs (KMT2A-H)	LSD1,2 (KDM1A-B) JARID1B/PLU1 (KDM5B)
		me2	Early origins	Not reported.	MLLs (KMT2A-H) NSD2-3 (KMT3F-G) SET7/9/D7 (KMT7)	LSD1,2 (KDM1A-B) JARID1B/PLU1 (KDM5B)
		me3	Early origins	CDC45 binding inhibition [20].	MLLs (KMT2A-H) SMYD3 (KMT3E) PRDM9 (KMT8B)	JARID1B/PLU1 (KDM5B) LOXL2
	9	me1	Early origins	Heterochromatin assembly [48].	SETDB1 (KMT1E) PRDM3,16 (KMT8E-F)	JMJD1A-C (KDM3A-C) LSD2 (KDM1B) JMJD2A-D (KDM4A-D)
		me2	Mid phase origins	Heterochromatin assembly.	G9a/EHMT2 (KMT1C) GLP/EHMT1 (KMT1D) PRDM2,8 (KMT8A,D) ASH1L (KMT2H)	JMJD1A-C (KDM3A-C) LSD2 (KDM1B) JMJD2A-D (KDM4A-D)
		me3	Late origins	Heterochromatin assembly [67].	SUV39H1/2 (KMT1A-B) PRDM2 (KMT8A)	JMJD2A-D (KDM4A-D)
	27	me1	Not reported	Replication Elongation [23].	EZH2 (KMT6) NSD2 (KMT3G)	JHDM1D (KDM7A) PHF8 (KDM7B)
	36	me1	Early origins	Regulation of CDC45 binding [21].	NSD1 (KMT3B) SMYD2 (KMT3C)	FBXL11,10/JHDM1A,B (KDM2A,B)
		me2	Not reported	Not reported.	NSD1 (KMT3B) SMYD2 (KMT3C)	FBXL11,10/JHDM1A,B (KDM2A,B) JMJD2A-D (KDM4A-D) JMJD5 (KDM8)
		me3	Late origins	Regulation of CDC45 binding [21].	SETD2 (KMT3A)	JMJD2A-D (KDM4A-D)
	56	me3	Late origins? (heterochromatin)	Not reported.	SUV39H1,2 (KMT1A-B)	JMJD2E (KDM4L)
	79	me1	Not reported	Origin licensing [22].	DOT1L (KMT4)	Unidentified
		me2	Origins		DOT1L (KMT4)	Unidentified
H4	20	me1	Not reported	Origin licensing [17].	SET8/PR-SET7 (KMT5A) NSD1,2 (KMT3B,G) ASH1L (KMT2H)	JHDM1D (KDM7A) PHF8 (KDM7B)
		me2	Late origins? (heterochromatin)	ORC recruitment [14,16]. Heterochromatin assembly [67].	SUV4-20H1/2 (KMT5B-C) NSD1,2 (KMT3B,G) ASH1L (KMT2H)	JHDM1D (KDM7A) PHF8 (KDM7B)
		me3	Late origins? (heterochromatin)		SUV4-20H1/2 (KMT5B-C) ASH1L (KMT2H)	Unidentified

to chromatin [14]. Peptide arrays also identified that H3K9me3 and H3K27me3-containing peptides can interact with ORC components [15]. Importantly, in line with in vitro results, chromatin immunoprecipitation (ChIP) experiments show an increase in the H4K20me2 signal at defined origins accompanied by higher occupancy of hORC1 relative to adjacent sequences. Conversely, upon depletion of Suv4-20, the enzyme that imposes this mark, ORC subunit occupancy decreased [16]. Thus, first hints into a possible role for H4K20me2 are provided as a means to regulate origin recognition with the example of the recruitment of ORC components. The detailed mechanisms await further experimental work and in vivo assays will be needed to validate a functional relevance.

2.2. Origin licensing

Origin licensing is characterized by the formation of the preRC complex. While, to date, a direct role for histone methylation in recruiting the preRC is missing, the coincidence of the onset of licensing with an increase in H4K20me1 at origins in mammalian cells is intriguing. Next, PR-Set7, the methyltransferase responsible for H4K20me1 [17], is degraded, preventing re-replication [16,18]. The importance of PR-Set7 (also known as Set8 or KMT5a) has been demonstrated by tethering it to a specific genomic locus, this in turn lead to the loading of the pre-RC complex in a manner dependent on Suv4-20, which catalyzes the H4K20me3 reaction (Fig. 1) [18]. Thus, the dynamics of H4K20 methylation is important for the proper selection of origins in mammalian cells. How this kind of selection can be achieved in yeast, which lacks PR-Set7, should be examined to determine if H4K20 methylation mediated by another methyltransferase may act in the same pathway or if a distinct mechanism is at work. In this respect, one should note that in yeast, a role for H3K4me2 was proposed based on a genetic screen to identify histone modifications important in DNA replication [19]. Perturbation of either the enzymes responsible for the marks or mutating H3K4 to H3R4 resulted in minichromosome maintenance defects [19]. Thus, future work should elucidate further whether these particular marks show a conserved role across species at the time of replication and whether they act alone or in combination with other histone modifications.

2.3. Origin firing

Origin firing requires the binding of CDC45 to the preRC. Notably, methylation on H3K4 and H3K36 has been implicated in CDC45 recruitment (Fig. 1). This was documented through in vitro peptide-binding assays where unmodified H3 peptides efficiently pulled down CDC45 from nuclear lysate and recombinant CDC45, whereas the H3K4me3 peptide did not [20]. Consistently, ChIP analysis of H3K4me3 at the B-globin replication origin in Jurkat cells shows that H3K4me3 does not correlate with CDC45 enrichment [20]. Notably, in cells expressing a catalytic dead mutant of MLL, the H3K4me3 methyltransferase, CDC45 does accumulate at this origin [20]. Thus, H3K4me3 could prevent the binding of CDC45. In yeast, genome-wide studies show that an increase in H3K36me1 correlates with CDC45 binding and this mark is enriched in early replicating genes, whereas H3K36me3 is present in late-replicating genes, suggesting that this mark may delay CDC45 binding (Fig. 1) [21]. In addition, genome-wide studies in human K562 cells showed an enrichment of H3K79me2 at replication

initiation sites [22]. Interestingly, the modulation of the cellular levels of DOT1L, the enzyme that imposes this mark, impacts the extent of replication in different organisms. Whereas depletion of human DOT1L leads to some DNA regions to replicate more than once per cycle [22], in *Trypanosoma brucei*, over-replication is observed upon DOT1A (a DOT1L homologue) overexpression [22]. Altogether, these studies strengthen the notion that roles for methylation in regulating replication may be species-specific and they underline the fact that the context in which these marks are present is also important.

2.4. Replication elongation

Following origin firing, the eukaryotic replisome promotes replication elongation. A role for H3K27 methylation has been assigned in tetrahymena cells during this time [23] (Fig. 1). Notably, PCNA, a protein that can bind several factors at the replication fork, interacts in particular with TRX1, the methyltransferase enzyme that catalyzes H3K27me1. Upon deletion of this enzyme, the levels of H3K27me1 are reduced as shown by mass spectrometry analysis and single stranded DNA (ssDNA) accumulates, indicating a perturbation of replication [23]. Consistently, mutation of K27 to glutamine mimicked the phenotype of the TRX1 deletion [23]. Further experiments should aim to elucidate the mechanistic role of H3K27me1 in replication elongation, including whether this mark is present ahead or behind the replication fork, and how its dynamics impacts the elongation process.

To summarize, histone lysine methylation states at defined residues have been implicated in regulating different steps in DNA replication. While many of these early studies exploit in vitro approaches or present correlative studies linking methylation with the presence and/or absence of replication origin binding proteins, they point to a role of histone modifications in regulating replication. Future work will be needed to try to understand the mechanisms by which methylation impacts replication processes, including the role of the opposing activities of methyltransferases and demethylases that establish, remove, and maintain these marks to define the chromatin landscape.

3. Inheritance of histone methylation marks after passage of the replication fork

Methylation of distinct histone lysine residues has been implicated in defining chromatin domains, including regions of active transcription, silenced regions, and heterochromatin [4,24]. How this information can be transmitted (or not) through multiple cell divisions to maintain the gene expression profiles that impact cell function has raised increasing interest [25–27]. In this section we discuss specifically how methyl marks may be propagated during replication first by describing the contribution of recycled parental histones and the impact of depositing newly synthesized histones. We then discuss the propagation of H3K27 methylation, an important mark in gene silencing [28,29], by the polycomb group (PcG) proteins. Finally, we describe mechanisms that participate in dynamically maintaining defined chromatin regions, using heterochromatin as a model chromatin domain.

3.1. Parental and newly synthesized histones: setting the stage for chromatin inheritance?

In standard cultured cells, current data support the view in which parental $(H3–H4)_2$ tetramers randomly segregate to the nascent strands, bringing along their modifications (reviewed in [30,31]). Notably, since H2A–H2B dimers are more dynamic, to date they have been largely ignored in their capacity to provide a means for parental H2A–H2B inheritance during replication. The most recent analysis focusing on H3 variants used stable isotope labeling of amino acids in cell culture (SILAC)-based mass spectrometry experiments to visualize whether endogenous and tagged histone dimers mix after replication. These experiments could reveal that the replacement variant H3.3 does mix by 10–20%, but they could not detect a similar mixing with the replicative histone variant H3.1 [32,33]. However, whether this is due to the nature of the variant or to the nature and dynamics of the underlying associated genomic sequences with respect to their transcriptional state is unknown. Interestingly, cell-type specific marks may play a role in this process [35], and this could impact the inheritance of certain marks at defined regions. Finally, whether the parental $(H3–H4)_2$ tetramers present symmetric histone modifications (where both copies of H3 and H4 feature identical marks) or asymmetric modifications [34], could also impact the propagation of the chromatin landscape.

Newly synthesized histones generally feature distinct marks, such as H4K5K12 diacetylation and H3K9me1 [35–37], although it is important to note that not all histones presenting these marks are necessarily newly synthesized. Interestingly, the histone variants H3.1 and H3.3 exhibit a differential methylation pattern, where H3.1 is enriched in the H3K9me1 mark [37], and this might impact further modifications at defined loci. The coordination of the deposition of newly synthesized histones and the recycling of parental histones has major implications in reestablishing the chromatin landscape. In this respect, histone chaperones, proteins that escort histones and are involved in their transfer without necessarily being part of the final product [38], represent key candidates for recycling histones. In the context of replication, one should stress the importance of two histone chaperones with major roles in handling histones at the replication fork (for reviews see [38,39]). First, chromatin assembly factor-1 (CAF-1), which deposits the replicative histone variant H3.1–H4 in a manner coupled to DNA synthesis [40], and second, anti-silencing function 1 (ASF1), that is found associated with the helicase complex and proposed to coordinate the recycling and de novo deposition of newly synthesized histones [41,42].

Once the chromatin is reassembled, the methyl marks on the parental and newly synthesized histones may recruit chromatin binding proteins to further modify the histones. Notably, some histone lysine methyltransferases, including MLL, SetDB1, Suv39H1/2, Suv4-20H1/2 and others, contain structural domains that recognize and bind methylated lysines, such as chromo, tudor, and PHD domains (reviewed in [24]). Some of them even exist in complexes with the enzymatic activity required for further modifications. One example is the EED component of the PRC2 complex, which recognizes and binds H3K27me3, to promote PRC2 activity to methylate neighboring nucleosomes [32,43]. Further, Suv39h1, an H3K9 methyltransferase, recognizes and binds H3K9me3 through its chromodomain [44]. Suv39h1 can then methylate neighboring histones, converting its preferred substrate H3K9me1 to the di and trimethylated state [37,45–47]. Enzymes may also modify non-nucleosomal histones in transit, including parental histones evicted ahead of the fork and newly synthesized histones prior to deposition. Indeed, SetDB1 monomethylates soluble H3K9 [48], which, depending on the localization of the deposited histones, may prime particular genomic regions for further modifications [48] or recruit a particular partner to initiate a biological response. Recently, additional enzymes, termed the PRDM enzymes, have been implicated in monomethylation of H3K9 [49]. It will be important to delineate how the choice of the enzyme impacts the fate of histones and/or whether the enzymes act redundantly. Thus, defining how and when the H3K9me1 mark is established, which enzymes are responsible for these modifications, and how histones bearing this mark are targeted to particular chromatin domains will be major goals to shed light on how histone marks can influence the inheritance of chromatin domains.

3.2. Polycomb Repressive Complexes: new players in epigenetic inheritance?

The polycomb group (PcG) proteins, initially identified in *Drosophila* [50,51] and conserved in metazoans [52,53], have been implicated in gene silencing (for a review the reader is referred to [54]). Polycomb repressor complex 2 (PRC2) methylates H3K27 to H3K27me3, a hallmark of silenced genes, while PRC1 recognizes and binds H3K27me3 through the chromodomain of the Polycomb (Pc) protein [28,29,55,56].

Immunofluorescence approaches enabled the detection of an increased PRC1 assembly on chromatin from mitosis to G1, suggesting that the complex may be unloaded from chromatin during S phase [57]. However, in a cell-free DNA replication system, the binding of PRC1 to chromatin can tolerate passage of the replication machinery, potentially through an interaction with the DNA itself [58]. Additionally, this in vitro system found that the PRC1 protein posterior sex combs (PSC) self-associates [28,43,59]. The latter study suggests a model where the cell exploits the dimerization of PSC to bridge PRC1 bound to parental chromatin to PRC1 associated with the daughter strands. This would thus permit passage of the replication fork while ensuring the propagation of PRC1 occupancy at defined chromatin regions [28,43,59].

Reestablishing H3K27me3 on the duplicated chromatin depends on the methyltransferase E(z), a member of the PRC2 complex [43]. Several studies exploiting mass spectrometry approaches show that H3K27me3 appears stable following replication, supporting the view that parental histones and marks are recycled [60,61]. However, studies in *Drosophila* have challenged this view. Using immunofluorescence approaches in *Drosophila* embryos, the authors could detect PcG protein E(z) on the newly synthesized DNA without detection of H3K27me3 [63,64]. They proposed a model where the complexes comprising the enzymatic activities, rather than the methyl marks themselves, are propagated at defined chromatin regions to reestablish the methylation state following replication fork passage. However, most recently, the characterization of histone marks on newly synthesized DNA in human cells by capturing nascent chromatin could reveal unambiguously that H3K27me3 levels did not change when compared to the levels observed in mature DNA [62]. Thus one should consider that in *Drosophila* either H3K27me3 was lost through degradation or catalytic demethylation during the recycling of parental histones, or simply fell below the detection limit of the employed assay conditions. Finally, other studies demonstrate that H3K27me3 and even H3K9me3 replenishment occurs gradually through the cell cycle, completing its reestablishment at post-mitotic G1 phase [57,65,66]. Thus, there is still some debate concerning the exact fate of these modifications during and after replication, and a careful examination under similar experimental conditions in different organisms is needed to clarify the issue.

3.3. Histone lysine methylation: setting the stage for heterochromatin reestablishment?

Heterochromatin domains are transcriptionally silent regions of the genome enriched in repetitive sequences and transposable elements [67]. This chromosomal landmark, featuring methylation marks such as H3K9me3 and H4K20me3 and is enriched with the chromatin binding protein heterochromatin protein 1 (HP1) (Fig. 2A), must be

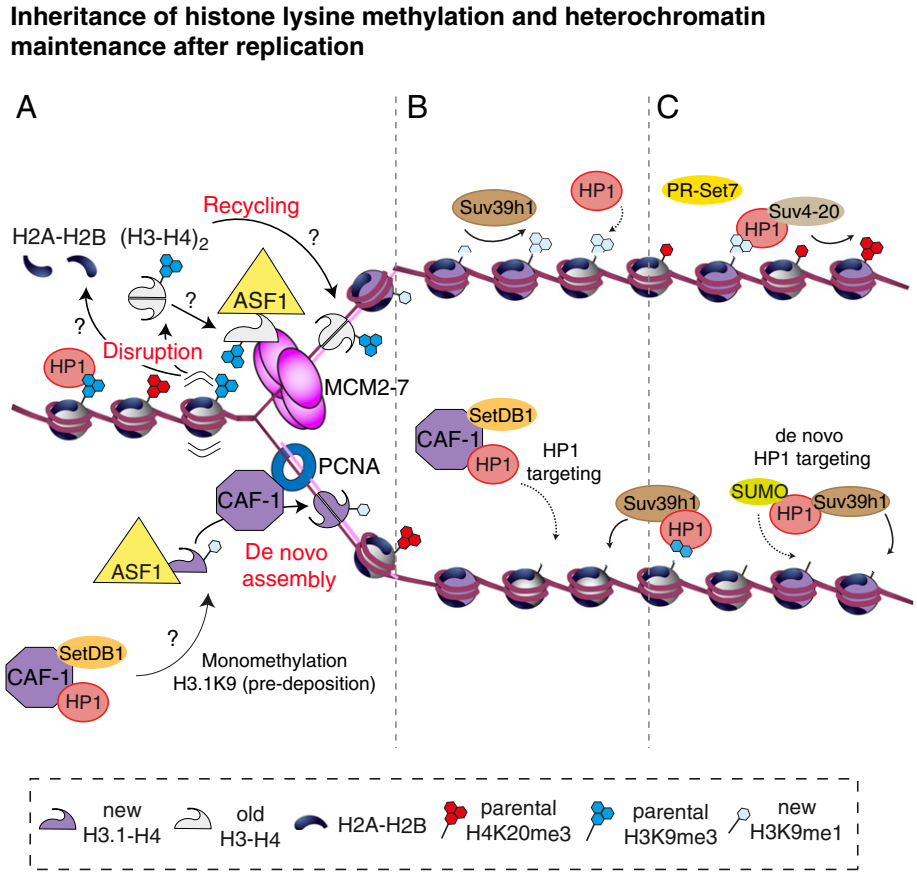

Fig. 2. Propagation of lysine methylation marks and heterochromatin maintenance after replication. A. Ahead of the replication fork, nucleosomes are transiently disrupted, and parental H3–H4 histones are handled by the histone chaperone anti-silencing function 1 (ASF1). Evicted $(H3–H4)_2$ tetramers may proceed through a dimeric state. Indeed, ASF1 is in a complex with the MCM helicase, which is mediated by a histone (H3–H4) dimer bridge. The parental histones are likely randomly distributed to the daughter strands (grey nucleosomes). For the deposition of newly synthesized histones, ASF1 donates H3.1–H4 dimers to CAF-1, and deposition is coupled to DNA synthesis through an interaction with PCNA. A complex containing CAF-1, SetDB1, and HP1 may be involved in monomethylating H3.1–H4 prior to deposition, a reaction that occurs on newly synthesized or parental histones in transit. B. (top) Histones presenting H3K9me1 are substrates for the methyltransferase Suv39h1, which catalyzes the methylation to the trimethylated state. (bottom) The CAF-1, SetDB1, and HP1 complex may participate in targeting HP1 to heterochromatin domains. Solid arrows represent methylation reactions, dotted lines represent the targeting of protein–protein interactions. C. (top) Pr-Set7 (or the methyltransferase Set8) catalyzes monomethylation of H4K20, a substrate for Suv4-20, which re-establishes the heterochromatin mark H4K20me3. (bottom) HP1 interacts with H3K9me3 nucleosomes, and the interaction with Suv39h1 provides a feed-forward loop to spread the heterochromatin mark to neighboring nucleosomes. Independently of H3K9me3, HP1 can also be targeted de novo to heterochromatin sites by a sumoylation mechanism. Solid lines represent enzymatic reactions, dotted lines represent targeting protein–protein interactions.

maintained through multiple cell divisions to maintain genome stability (reviewed in [26,29,67,68]). Here we use it as a case study on how methylation patterns can be stably inherited to maintain particular states in defined chromatin domains.

Several mechanisms are in place to maintain heterochromatin offering a robust means to back up one another in case one pathway is perturbed. First, de novo histone deposition of H3.1–H4 featuring H3K9me1 is the preferential substrate for Suv39h1 to the H3K9me3 state, leading to the recruitment of HP1 [48] (Fig. 2B). Interestingly, the histone chaperone CAF-1 exists in a complex with SetDB1, a methyltransferase implicated in catalyzing H3K9me1 on soluble histones, and HP1 [48]. In addition, the PRDM enzymes have also been involved in the monomethylation of soluble H3K9 [49]. This offers a possible mechanism for heterochromatin formation from de novo histone deposition where newly synthesized histones featuring H3K9me1 are deposited, further modified to H3K9me3, which then recruits HP1 (Fig. 2B) [69]. A recent work in *Caenorhabditis elegans* showed a similar stepwise mechanism to establish H3K9me3 on heterochromatin [70]. Like H3K9me3, H4K20me3 could result from a stepwise mechanism, in which PR-Set7/Set8 monomethylates H4K20 [71,72] and then Suv4-20 converts H4K20me1 to H4K20me2/3 [73]. However, while PR-Set7/Set8 interacts with PCNA through a conserved motif [74], its specific recruitment to sites of heterochromatin replication has not been elucidated yet. In contrast, Suv4-20 interacts with the protein HP1 [73], providing a possible mechanism for the propagation of this modification on the replicating heterochromatin (Fig. 2C). Secondly, recycling parental histones featuring H3K9me3 may recruit HP1 through its chromodomain (Fig. 2C) [46,47] to further recruit Suv39h1 [45] and modify neighboring nucleosomes. Finally, sumoylation of HP1 has been put forward as a mark that leads to de novo recruitment of HP1 at heterochromatin sites, independent of H3K9me3 status, as observed in cells lacking Suv39h1 and void of H3K9me3 (Fig. 2C) [75]. In addition to H3K9me3 and H4K20me3, recent studies have suggested that H3K56me3 is a novel mark of heterochromatin. Immunostaining analyses on HeLa cells showed that the H3K56me3 mark localizes to pericentric heterochromatin and co-localizes with H4K20me3 [76]. However, further studies are necessary to define the role of this mark as well as its inheritance mechanism in heterochromatin.

We have reviewed the links between lysine methylation of histones H3 and H4 and chromatin replication, particularly in three aspects: the role of lysine methylation in the initiation of DNA replication, pathways to propagate lysine methylation marks following replication, and the pathways to reestablish the characteristic lysine methylation landscape of heterochromatin. In each aspect, the degree of histone lysine methylation on defined residues plays various roles. Thus, the current challenge is to have an in-depth comprehension of the function of histone lysine methylation on chromatin replication, to elucidate whether histone methylation patterns affect replication directly or indirectly, and to unveil how these marks are inherited and/or re-established in other chromatin domains. It is critical to have a better understanding of the handling of both parental and newly synthesized histones and how different modifications may prime the chromatin for additional modifications by the methylases and demethylases. The development of innovative approaches, including combining mass spectrometry approaches, novel biochemical and structural tools, and in vivo assays will be critical to provide new insight into the mechanisms behind the role of histone modifications in regulating chromatin replication and the impact on chromatin inheritance.

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