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REVIEW

Nucleosome assembly and epigenetic inheritance

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ABSTRACT

In eukaryotic cells, histones are packaged into octameric core particles with DNA wrapping around to form nucleosomes, which are the basic units of chromatin (Kornberg and Thomas, 1974). Multicellular organisms utilise chromatin marks to translate one single genome into hundreds of epigenomes for their corresponding cell types. Inheritance of epigenetic status is critical for the maintenance of gene expression profile during mitotic cell divisions (Allis et al., 2006). During S phase, canonical histones are deposited onto DNA in a replication-coupled manner (Allis et al., 2006). To understand how dividing cells overcome the dilution of epigenetic marks after chromatin duplication, DNA replication coupled (RC) nucleosome assembly has been of great interest. In this review, we focus on the potential influence of RC nucleosome assembly processes on the maintenance of epigenetic status.

KEYWORDS nucleosome assembly, epigenetic inheritance, DNA replication coupled

INTRODUCTION

An adult animal contains over 200 different cell types, each of them has specialized structure and distinct physiological function (Allis et al., 2006). With a few exceptions, all of these cells carry the same genetic information encoded by DNA. The identity of a given cell type is determined by its characteristic gene expression profile. During development and adult homeostasis, it is important to faithfully maintain this state after each cell division. Any unscheduled compromise might lead to developmental disorder or disease. Developmental studies in *Drosophila* in 1960s and 1970s demonstrated that cells can memorize their committed fates, even after being removed from their original environment and being cultured for several cell divisions (Hadorn, 1968; Eissenberg, 2006). For example, imaginal disc cells from *Drosophila* larvae can be cultured *in vitro* and undergo cell proliferation without differentiation. Moreover, these cultured cells can be transplanted back into larvae and differentiate into appropriate structures (Hadorn, 1968). This suggests that cells can remember their identity and transmit this information to the daughter cells during cell division. However, the underlying mechanism remains poorly understood.

Each nucleosome contains 146 bp of DNA wrapping in 1.75 left-handed superhelical turns around a histone octamer (Luger et al., 1997). Histone octamer is organized with a central H3/H4 tetramer flanked by two H2A/H2B dimers at either side. Histone N- and C-terminal tails protrude out of the DNA-protein globular core, which enable their modification sites to be accessible to a series of chromatin regulators (Luger et al., 1997). Long before understanding the subtlety of chromatin modifications, it was already proposed that chromatin structure could impact transcription (Allfrey and Mirsky, 1964). Thus potentially, chromatin modifications have regulatory roles in tissue specific gene expression (Paul and Gilmour, 1968). However, the direct connection between chromatin modification and transcriptional regulation was not established until the milestone discovery that characterized *Tetrahymena* homologue of yeast transcriptional regulatory protein Gcn5 as a histone acetyltransferase (Brownell et al., 1996). Since then, molecular mechanism underlying epigenetic control of chromatin structure was explosively unveiled (reviewed in Henikoff et al., 2004; Shilatifard, 2006;

Shahbazian and Grunstein, 2007; Mosammaparast and Shi, 2010). Briefly, DNA is covalently modified by methylation on cytosine; histones are also post-translationally modified by acetylation, methylation, phosphorylation and ubiquitination, these modifications work in concert as a "histone code", which can be deciphered by a variety of modification binding proteins (Jenuwein and Allis, 2001); on the other hand, exchange of histone variants independent of DNA replication provides another layer of epigenetic regulation (Henikoff et al., 2004). Now, it is generally accepted that these epigenetic marks are necessary for the maintenance of chromatin signatures, including those regulating gene expression pattern (Polycomb silencing, position effect variegation and genomic imprinting, etc.), and those maintaining essential chromatin architectures such as centromere and telomere.

Since maintenance of epigenetic information is crucial for cell fate restriction during development, epigenetic marks are required to be appropriately transmitted from generation to generation during mitotic divisions. In mitotic cell cycle, DNA replication during S phase poses an important question of how, once duplicated, epigenetic signatures are re-established on nascent chromatin. As a result of DNA polymerase passage, the structure of paternal nucleosomes (for example, the intra- and inter-nucleosome combination of histone modifications) is disrupted; old histones should be re-organized on newly synthesized DNA. At the same time, canonical histones are produced tightly coupling to DNA synthesis (Marzluff et al., 2008), fulfilling the gaps among parental histones. Therefore, pre-existing marks are diluted due to incorporation of new histones. In order to make the chromatin marks inheritable, organisms should evolve a refined mechanism to coordinate the recycling of paternal histones and the deposition of newly synthesized histones.

In this review, we will discuss the role of replication coupled chromatin assembly in restoration of epigenetic states, as well as the potential ways in which chromatin assembly can contribute to the inheritance of epigenetic marks.

EARLY INVESTIGATIONS ON RC NUCLEOSOME ASSEMBLY AND THEIR CONTRIBUTIONS TO UNDERSTANDING OF EPIGENETIC INHERITANCE

Early investigations on the fate of histones during RC nucleosome assembly addressed three questions that are fundamentally important to current concepts of epigenetic inheritance. (1) When disassembled from pre-replicative nucleosomes, parental histones, especially $(H3/H4)_2$ tetramers, remain associated with DNA and they rapidly reassemble into nucleosomes on replicated DNA. (2) Pre-existing histones are randomly distributed to both of the nascent DNA strands in cluster. (3) The majority of parental $(H3/H4)_2$ tetramers remain intact during DNA replication.

$(H3/H4)_2$ tetramers associate with DNA during replication fork passage

As six major lysine methylation sites are present on either H3 (Lys4/9/27/36/79) or H4 (Lys20), mechanism that restricts massive exchanges between parental H3/H4 and free histones during replication is vitally important to epigenetic inheritance, as it protects the existing chromatin modification pattern from disruption. It took many laboratories a decade starting from the mid 1980s to address this question. The earliest clue came from experiments using the SV40 minichromosome replication system. Using electron microscopy to study psoralen cross-linked replicating SV40 chromatin, Sogo et al. found that replication fork moves up to, and possibly penetrates into, the unreplicated parental nucleosome ahead; nascent nucleosomes assembled on the replicated DNA at distances of 225 bp and 285 bp from the branching point separately for the leading and lagging strands (Sogo et al., 1986). This suggests that a full set of core histones are rapidly reassembled following replication advancement. The first direct evidence for the association of parental histones with replicating DNA was obtained using a defined prokaryotic chromatin replication system. Chromatin were assembled with purified core histones and plasmid DNA, and then replicated by replication proteins purified from bacteriophage T4. Core histones reassemble on replicated DNA even in the presence of excessive nucleosome-free competitor DNA (Bonne-Andrea et al., 1990). This was further confirmed with the SV40 minichromosomes isolated from lytically infected cultured cells (Krude and Knippers, 1991; Sugasawa et al., 1992) or *in vitro* assembled chromatin containing SV40 replication origin (Randall and Kelly, 1992). These studies also postulated that the pre-existing histones remain bound during the replication process. However, contradictions remain: Sugasawa et al. (1992) and Randall et al. (1992) concluded that old octamers do not dissociate from replicating DNA. However, the ratio of competitor DNA to chromatin DNA (0.3–1) in these experiments was low. In contrast, data from some other groups suggest the parental octameric structures are disrupted during the passage of replication fork (Sogo et al., 1986; Fotedar and Roberts, 1989), and some H2A/H2B dimers dissociate from replicating DNA (Jackson, 1987, 1990). Finally, using an improved *in vitro* replication method, Gruss et al. clarified the debate and provided further insights (Gruss et al., 1993). The authors concluded that, a subnucleosomal particle, probably the $(H3/H4)_2$ tetramer is the transfer unit, which, then assembled with two H2A/H2B dimers to form an intact nucleosome. In addition, 5–10 folds of competitor DNA during replication traps histones transferred to daughter strands, suggests that the histones may only loosely associated with replicating DNA (Gruss et al., 1993). However, a few open questions remain, such as what are the chaperon factors that assist the disassembly/reassembly process of the parent nucleosomes

and how they coordinate with chaperons facilitate the deposition of newly synthesized histones.

Parental histones are randomly distributed to newly synthesized DNA

In order to transmit the pre-existing marks to newly deposited histones, the establishment of modifications on new histones should be, at least to some extent, guided by pre-existing modifications on parental histones. Thus, the way in which parental histones are distributed to daughter DNA strands might determine the inheritability of histone marks. One early model proposed that the old histones cover the leading strand of the replication fork while newly synthesized histones complex with the lagging strand (Seale, 1976; Riley and Weintraub, 1979; Seidman et al., 1979). However, after a period of debate, the field has finally reached to the conclusion (McKnight and Miller, 1977; Cusick et al., 1981, 1984; Fowler et al., 1982; Pospelov et al., 1982; Annunziato and Seale, 1984; Jackson and Chalkley, 1985) that parental histones are randomly distributed to both leading and lagging strands. Most of the early studies on parental histone segregation were carried out in cycling cells with the treatment of protein synthesis inhibitors, such as cycloheximide and puromycin. Subsequent experiments were based on nuclease digestion followed with electrophoresis or sedimentation (Pospelov et al., 1982; Annunziato and Seale, 1984; Jackson and Chalkley, 1985) or based on electron microscopy (McKnight and Miller, 1977) to analyze so called “cycloheximide chromatin”. Taken together, these evidences demonstrated that parental histones are dispersively segregated to both copies of newly replicated DNA. The same question was also investigated in a cell free system for SV40 minichromosome replication (Cusick et al., 1984; Krude and Knippers, 1991; Randall and Kelly, 1992). In the absence of free histone supply, parental histones segregate to both of the nascent DNA strands, in consistence with the studies performed in living cells treated with protein synthesis inhibitors.

H3/H4 segregated to daughter strands as tetramers

In the past three decades, large amount of literature can be found on the discussion: how pre-replicative $(H3/H4)_2$ tetramers are segregated during replication. One model concluded that old H3/H4 are transferred to nascent DNA as tetramers, in contrast, the other one proposed that parental H3/H4 tetramers dissociate into dimers, then each old H3/H4 dimer assembles with a newly synthesized H3/H4 dimer. In recent years, the debate on this seemingly trivial issue attracted increasing attention for its potential significance in the inheritance of histone modifications (reviewed in Nakatani et al., 2004; Annunziato 2005; Henikoff and Ahmad, 2005; Hake and Allis, 2006; Groth et al., 2007; Martin and Zhang, 2007; Probst et al., 2009). If the $(H3/H4)_2$ tetramer split model is correct, it provides a perfect model for the transmission of histone modifications, in which new H3/H4 dimer could use the epigenetic information at the old H3/H4 dimer within the same nucleosome as a template to precisely establish its modifications, thus faithfully restore the information of parental nucleosome at exactly the same genomic region. Historically, there are good evidences for the H3/H4 tetramer non-split model. In 1980, using the slime mold *Physarum* as a model, Prior et al. (1980) examined the long term stability of H3-H3 interactions. Data showed that, even after five rounds of DNA replication, the original H3-H3 interaction can still be detected, indicating that $(H3/H4)_2$ tetramers are stable during multiple cell generations *in vivo* (Prior et al., 1980). Ten years after this discovery, Jackson studied the fate of $(H3/H4)_2$ tetramers in proliferating mammalian cultured cell via density labeling and sedimentation approach (Jackson, 1990). The author concluded that in contrast to the massive exchange of H2A/H2B dimers, most $(H3/H4)_2$ tetramers do not split even after several rounds of DNA replication. Yamasu and Senshu (1990) also studied the same question by dense labeling in a method different from Jackson’s, which provided similar conclusion. Although most of these studies favored the H3/H4 tetramer non split model, the recent discoveries found that H3/H4 histones deposit into chromatin as dimers, rather than as tetramers (Tagami et al., 2004; English et al., 2005; Benson et al., 2006). Crystal data clearly showed the heterotrimeric structure of Asf1-H3/H4 (Natsume et al., 2007). In addition, Asf1 can disassemble the preformed H3/H4 tetramers, thus potentially allowing the newly synthesized H3/H4 dimers to be paired with old H3/H4 dimers (Natsume et al., 2007). These results again raised the possibility of H3/H4 tetramer split model and spurred the debates. We reinvestigated the question by selectively purifying mono-nucleosomes containing one “old” histone H3.1 and determined that its partner histone H3.1 is also an old one by stable isotope labeling based quantitative mass spectrometry (Xu et al., 2010). This study was variant specific and distinguished the “old” and “new” histones with full resolution, thus effectively put the H3/H4 tetramer splitting model to an end, at least for the canonical histones. Therefore, if there is any templated modification copying event, it could only occur by copying methylation marks from neighboring nucleosomes.

MOLECULAR MECHANISM OF REPLICATION COUPLED CHROMATIN DISASSEMBLY AND REASSEMBLY

Developmentally regulated gene expression is achieved by heritable changes in chromatin structure during cell differentiation. In higher organism, refined mechanisms are evolved both to change chromatin structure and to maintain these structures to daughter cells. DNA replication disrupts chromatin organization and dilutes epigenetic marks, thus

provides a window of opportunity to implement these maintenances or changes between cell generations. Therefore, the molecular model underlying these processes is highly interesting. To address this question, the first priority is to understand how chromatin is assembled during DNA replication. Replication coupled chromatin duplication contains the process of chromatin disassembly ahead of replication fork and chromatin reorganization behind the fork. During replication fork progression, high order chromatin structure is destabilized in a stretch of approximately two nucleosomes in the parental chromatin (Gasser et al., 1996), and the first pre-replication nucleosome is disrupted (Sogo et al., 1986). The restoration of chromatin structure behind of replication fork can be divided into several steps. First, assembly begins with the incorporation of H3/H4 tetramer, which is followed by the addition of two H2A/H2B dimmers, to form a complete nucleosome structure (Jackson, 1987, 1990; Gruss et al., 1993). The incorporation of linker histone was detected 450–650 bp after replication fork passage (Gasser et al., 1996), enabling further folding of higher order structures. The molecular machinery involved in these processes will be discussed in the following section.

Disassembly of chromatin structure ahead of replication fork

Replication of eukaryotic genome requires various replication factors to be in close contact with DNA, it is hard to think all of these processes can occur in the nucleosome context. In a defined *in vitro* replication system, the disassembly of nucleosome can be achieved by the progression of replication fork itself (Sugasawa et al., 1992), either due to unwinding of DNA helix by MCM2–7 helicase complex or the moving of replication machinery. However, like many other chromatin based processes such as transcription and DNA repair, replication coupled nucleosome disassembly is believed to be facilitated by ATP dependent chromatin remodeling factors and histone chaperons *in vivo*.

Several ATP dependent chromatin remodeling complexes are thought to play important role in disassembly of pre-replicative chromatin. ACF1-SNF2h was reported to be essential for the replication of heterochromatin in cultured mammalian cells (Collins et al., 2002). Other evidence showed that ISWI type nucleosome remodeling complex SNF2h is recruited to replication fork by WTSF, RNAi mediated knock down of SNF2h or WTSF caused reduction of DNA replication efficiency during S phase (Poot et al., 2004). In *S. cerevisia* INO80 complex is enriched in replication origins and stalled replication forks under replicative stress (Papamichos-Chronakis and Peterson, 2008; Vincent et al., 2008). Furthermore, mutation of INO80 leads to loss of replisome proteins under replicative stress (Papamichos-Chronakis and Peterson, 2008).

Disassembly of pre-replicative nucleosome is thought to be accomplished by dissociation of H2A/H2B dimmers from nucleosomes followed by the removal of $(H3/H4)_2$ tetramers. Multiple histone chaperones are found to be involved in this process. FACT complex which contains SPT16 and SSRP1 in human and Spt16 and Pob3 in yeast was first isolated as a transcription elongation factor (LeRoy et al., 1998; Orphanides et al., 1998). It has histone chaperone activity to both H2A/H2B and H3/H4 (Belotserkovskaya et al., 2003; Stuwe et al., 2008), and replaces H2A/H2B dimers from transcribing nucleosomes (Belotserkovskaya et al., 2003). In recent years, accumulating evidence showed that FACT also involves in chromatin replication. Using budding yeast as a model, VanDemark et al. (2006) showed FACT interacts with single strand binding protein RPA; Gambus et al. (2006) found FACT co-purifies with replication complex. These results were supported by genetic evidence that budding yeast FACT mutant is sensitive to replicative stress during hydroxyurea treatment (Schlesinger and Formosa, 2000). In mammals, FACT localizes to replication foci (Hertel et al., 1999; Tan et al., 2006) and interacts with MCM helicase (Tan et al., 2006). Another histone chaperone which potentially functions in removal of parental histones during replication is Asf1. Yeast Asf1 directly binds RFC *in vitro*, deletion of Asf1 results in reduced integrity of stalled replisome (Franco et al., 2005). In *Drosophila* S2 cells, Asf1 localizes to DNA replication foci during S phase, RNAi knockdown of dASF1 leads to accumulation of cells in S phase (Schulz and Tyler, 2006). In human cells, Asf1 binds MCM helicase complex, through H3 and H4. In the absence of Asf1, the proliferating cells represent delayed S phase progression and reduced DNA unwinding at replication fork (Groth et al., 2007). Furthermore, posttranslational modifications on chromatin were found in H3 co-purified with Asf1, suggests at least some of the H3/H4 dimers bound to Asf1 were evicted from pre-fork nucleosomes (Groth et al., 2007; Jasencakova et al., 2010).

Restoration of chromatin structure behind replication fork

The histones assembled onto replicated DNA are half from parental histone evicted ahead of replication fork and half from the pool containing newly synthesized histones. Although there is much work describing how parental nucleosomes are disassembled during replication as discussed above, little is known about the proteins responsible for the transfer of old histones. It is partially because the same histone chaperone may participate in multiple processes, including disassembly of the parental nucleosomes, transfer of the old histones and packaging of the nascent chromatin. Loss of function studies were performed on histone chaperones might be involved in delivery of old histones to nascent chromatin such as FACT and Asf1, and stalled replication fork advancement were observed. However, we can not distinguish whether these results are the consequence of impaired

nucleosome disassembly ahead of replication fork or impaired nucleosome reassembly behind the fork.

While our knowledge on histone chaperones related to the delivery of old histones is limited, the mechanism of depositing newly synthesized histone onto daughter DNA was extensively studied. Early attempts to uncover the molecular mechanism were carried out using *in vitro* replication of SV40 chromatin as a model. Efficient chromatin assembly on newly synthesized DNA requires the addition of nuclear factors. Smith et al. followed this RC nucleosome assembly activity in nuclear extract and isolated a three subunit protein complex termed as chromatin assembly factor I, CAF-I (Smith and Stillman, 1989). In cell free system, the loading of PCNA (proliferating cell nuclear antigen) is required for CAF-I mediated chromatin assembly (Shibahara and Stillman, 1999); furthermore, p150, the largest subunit of CAF-I complex directly interacts with PCNA (Shibahara and Stillman, 1999; Moggs et al., 2000). The interaction of p150 with histone H3/H4 through its KER and ED domains demonstrates CAF-1's histone chaperone activity (Kaufman et al., 1995). Moreover, histone H4 co-purified with CAF-1 are acetylated on K5 and K12, suggests they are newly synthesized in the cytoplasm (Kaufman et al., 1995; Verreault et al., 1996). *In vivo* data from different model organisms supported the conclusion of above *in vitro* experiments. Both RNAi mediated knockdown and overexpression of a dominant negative CAF-1 in cultured human cells result in defective assembly of newly replicated chromatin (Hoek and Stillman, 2003; Ye et al., 2003; Nabatiyan and Krude, 2004). Conditional depletion of CAF-1 p150 or p60 subunit in chicken DT40 cells leads to delayed S phase progression concomitant with slower DNA synthesis (Takami et al., 2007).

Acetylation appears to be a signature of newly synthesized histone H3 and H4. When synthesized in cytoplasm, H4 is acetylated at K5 and K12 (Sobel et al., 1995; Benson et al., 2006), these acetyl-groups are removed within 20 min after chromatin assembly (Taddei et al., 1999). In DT40 cells, HAT1 mediates H4 acetylation at these two sites (Barman et al., 2006). However, HAT1 is not required for replication coupled chromatin assembly *in vivo* (Barman et al., 2006). Thus, the role of K5 and K12 acetylation in nucleosome assembly remains to be uncovered. Newly synthesized H3 is acetylated at K56 in budding yeast (Masumoto et al., 2005) and mammals (Xie et al., 2009; Das et al., 2009). This modification is catalyzed by Rtt109 in budding yeast (Han et al., 2007; Tsubota et al., 2007), and requires the presence of histone chaperone Asf1 (Recht et al., 2006; Tsubota et al., 2007). H3 lysine 56 resides at the DNA entrance site in a nucleosome, the acetylation on this residue was reported to loosen the histone-DNA contact and increase nucleosome breathing (Neumann et al., 2009). Furthermore, H3K56ac serves a key role in RC nucleosome assembly. Li et al. (2008) found that acetylated K56 greatly increases the affinity of H3 to histone chaperone Rtt106 and CAF-1, thus promotes the nucleosome formation following DNA replication.

THE ROLE OF REPLICATION COUPLED NUCLEOSOME ASSEMBLY IN CHROMATIN RESTORATION

The biochemical characterization of factors involved in RC chromatin assembly offers an opportunity for genetically dissecting the relationship between nucleosome assembly and chromatin marking. Although the evidence is still limited, experiments based on RNA interference and knockout approaches *in vivo* provide some insights into the role of RC nucleosome assembly in epigenetic inheritance.

PCNA is an essential factor in DNA replication. It is loaded onto replicating DNA strand as heterotrimer in a ring like structure and then travels along with the replication fork. Yeast bearing mutant PCNA with reduced CAF1 interaction also displayed reduced gene silencing near telomere and silencing mating type loci, suggesting that PCNA participates in inheritance of not only DNA sequence but also epigenetic states (Zhang et al., 2000). To date, CAF-1 is the only known histone chaperone whose function is dedicated to the progression of DNA synthesis. Studies of CAF-1 in yeast led to the discovery of its role in the maintenance of silenced chromatin structures. Yeast strains bearing deletion mutations of CAF-1 subunits are viable, suggesting the role of CAF-1 in nucleosome assembly can be partially compensated by other pathways. However, these strains fail to maintain the repression states of distinct genome region such as telomere and mating type loci (Enomoto et al., 1997; Kaufman et al., 1997; Monson et al., 1997). Enomoto et al. provided further insight into the underlying mechanism using the yeast silent HM locus (HML) as a model (Enomoto and Berman, 1998), they found that CAF-1 is required for the maintenance of HML silencing during DNA replication, but not the re-establishment of silencing in alpha-factor arrested cells (Enomoto and Berman, 1998). Meijsing and Ehrenhofer-Murray (2001) discovered a genetic interaction between CAF-1 and yeast histone acetyltranferase SAS-1, which functions in promoting the silencing state on yeast telomere and mating type loci. This suggests that there might be some kind of connection between re-establishment of histone acetylation patterns and nucleosome assembly.

However, the heterochromatin structure in budding yeast is dramatically different from that of higher eukaryotes. Heterochromatin in higher organisms is much more complicated, with many additional components. Thus, whether nucleosome assembly is also important for maintaining epigenetic states in higher eukaryotes has attracted much attention. Temperature sensitive mutants of PCNA have been characterized in *Drosophila*, all of them strongly suppress PEV (position effect variegation), which reveals a role for insect

PCNA in chromatin assembly and modification (Henderson et al., 1994). Unlike *Drosophila* and mammals, in which CAF-1 is essential for survival, plants harboring deletions of either FAS1 or FAS2 (*Arabidopsis* CAF-1 p150 and p60) are viable (Kaya et al., 2001). Interestingly, knockout of *Arabidopsis FAS1* or *FAS2* disrupts the integrity of cellular organization in shoot apical meristem and root apical meristem. Functional study suggests *fas* mutants fail to maintain the expression of some meristem specific genes essential for its identity (Kaya et al., 2001), suggesting a critical role for CAF-1 in maintaining gene expression profile in proliferating cells.

THE POTENTIAL ROLE OF RC CHROMATIN ASSEMBLY IN EPIGENETIC INHERITANCE

As discussed above, replication coupled chromatin assembly not only packages all the newly produce DNA into nucleosome structure, but also contribute to the inheritance of chromatin state. How can nucleosome assembly affect the restoration of chromatin marks? Two potential mechanisms on distinct spatial and temporal circumstances can be envisioned: (1) restoring epigenetic marks right behind the replication fork in a replication-coupled manner; (2) establishing chromatin modifications during chromatin maturation independent of DNA replication.

Inheritance of epigenetic marks behind the replication fork?

During S phase, PCNA is loaded onto replicating DNA to facilitate the progression of both leading and lagging strand DNA polymerases. In recent years, in addition to the role in DNA synthesis, PCNA was found to coordinate the process of DNA synthesis, nucleosome assembly and inheritance of epigenetic marks. PCNA directly interacts with a series of chromatin modifying enzymes, and potentially recruits them to replication foci. These factors include CAF-1 (Shibahara and Stillman 1999; Moggs et al., 2000), HDACs (Milutinovic et al., 2002), ATP dependent chromatin remodeling complex WSTF-SNF2h (Poot et al., 2004), histone lysine methyltransferse PR-SET7 (Jørgensen et al., 2007; Huen et al., 2008) and DNA methyltransferase DNMT1 (Leonhardt et al., 1992; Chuang et al., 1997). CAF-1 is also reported to interact with chromatin factors, including MBD1 (methyl CpG-binding protein 1) and SETDB1 during heterochromatin DNA replication (Reese et al., 2003; Sarraf and Stancheva, 2004). In addition, DNMT1 directly interacts with histone H3K9 methyltransferase G9a, and recruits G9a to the replication foci (Estève et al., 2006). Using high resolution imaging techniques, Taddei et al. (1999) provided evidences that CAF-1 persists on newly replicated DNA after its synthesis for a period of more than 20 min, in consistence with the *in vitro* finding that CAF-1 assembles nucleosome post-replication (Shibahara and Stillman, 1999). In addition, a similar phenomenon was also observed on PCNA by fluorescent bleaching experiment (Sporbert et al., 2002). Hence, the association of PCNA and CAF-1 on replicated DNA could present a time window for synergistic restoration of chromatin structure and epigenetic marks. An attractive hypothesis is that, after traveling along with replication fork for a certain distance, PCNA and CAF-1 dissociate from the replisome and retain on the patch of newly synthesized DNA, during this period, they can serve as a landing pad for those chromatin modifying factors. As a consequence, histone deacetylases, histone methyltransferses, DNMT1 and ATP dependent chromatin remodeling factors can be recruited to this stretch of nascent chromatin, and work in concert to restore the epigenetic states.

Establishing chromatin modification during chromatin maturation independent of DNA replication?

Recently, stable isotope labeling based quantitative mass spectrometry provided new insights into the modifying events on newly deposited histones. Studies comparing the level of PTMs on new and old histones during cell cycle progression showed that while acetylation marks are quickly installed on new histones, the establishment of several methylation marks appears to be much slower (Pesavento et al., 2008; Scharf et al., 2009). Another experiment investigating the turnover rate of histone methylation suggests it takes more than one cell cycle to establish some of the methylation patterns (Zee et al., 2010). Thus, the re-establishment of certain methylation marks may occur during chromatin maturation independent of DNA replication.

Templated chromatin modification copying event?

In order to be inheritable, a pre-existing mark should be able to serve as a template to guide its establishment on newly synthesized histones. Potential examples of such copying events have been described for two histone modifications, H3K9me3 and H3K27me3. H3K9me3 at peri-centromeric heterochromatin is mainly catalyzed by histone methyltransferase SU(VAR)3-9 conserved from fission yeast (Clr4) to human (SU(VAR)3-9h1 and SU(VAR)3-9h2) (Rea et al., 2000; Nakayama et al., 2001; Peters et al., 2001). HP1 is a heterochromatin specific protein essential for maintenance of heterochromatin structure (Eissenberg et al., 1990). HP1 recognizes H3K9me3 with its chromodomain (Bannister et al., 2001; Lachner et al., 2001). In addition, HP1 interacts with Suv39 with its chromoshadow domain (Stewart et al., 2005). Moreover, the heterchromatic localization of HP1 and Suv39 are interdependent (Schotta et al., 2002). Therefore, although the H3K9me3 is also regulated by other mechanisms, such as RNAi in fission yeast (Volpe et al., 2002) and DNA methylation in mammals (Espada et al., 2004), the

positive feedback loop composed of Suv39 and HP1 does contribute to the recovery of its level after S phase. Another example is the self propagation of H3K27me3, a mark catalyzed by polycomb group protein PRC2, which is known as a regulator of cell memory (Schwartz and Pirrotta, 2007). Recently, using a reporter system in cultured human cells, Hansen et al. (2008) showed that H3K27me3 mediated gene silencing, once established, can be maintained independent of genetic elements through several cell divisions. They also found an *in vitro* binding between PRC2 and H3 tail trimethylated on lysine 27 (Hansen et al., 2008), which provides a possible explanation for the *in vivo* self maintenance. Combining structural biology, biochemistry and *Drosophila* genetics, Margueron et al. (2009) showed the potential mechanism of H3K27me3 transmission from mother cells to daughter cells. The PRC2 component EED binds H3K27me3, which results in the allosteric activation of PRC2 (Margueron et al., 2009). Moreover, *Drosophila* carrying EED mutations that are defective in binding H3K27me3 has reduced H3K27 methylation level and a polycomb phenotype (Margueron et al., 2009).

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