Epigenomic replication: linking epigenetics to DNA replication

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Summary

The information contained within the linear sequence of bases (the genome) must be faithfully replicated in each cell cycle, with a balance of constancy and variation taking place over the course of evolution. Recently, it has become clear that additional information important for genetic regulation is contained within the chromatin proteins associated with DNA (the epigenome). Epigenetic information also must be faithfully duplicated in each cell cycle, with a balance of constancy and variation taking place during the course of development to achieve differentiation while maintaining identity within cell lineages. Both the genome and the epigenome are synthesized at the replication fork, so the events occurring during S-phase provide a critical window of opportunity for eliciting change or maintaining existing epigenetic states. Cells discriminate between different states of chromatin through the activities of proteins that selectively modify the structure of chromatin. Several recent studies report the localization of certain chromatin-modifying proteins to replication forks at specific times during S-phase. Since transcriptionally active and inactive chromosome domains generally replicate at different times during S-phase, this spatiotemporal regulation of chromatin assembly proteins may be an integral part of epigenetic inheritance. *BioEssays* 25:647–656, 2003. © 2003 Wiley Periodicals, Inc.

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Abbreviations: CAF-1, Chromatin Assembly Factor-1; ACF-1, Alters chromosome function; WSTF, Williams Syndrome Transcription Factor; Asf-1, Anti-silencing factor; Nap-1/2, Nucleosome assembly protein; MBD2/3, Methyl CpG-binding proteins; PCNA, Proliferating cell nuclear antigen; RPA, Replication Protein A; DNMT, DNA Methyltransferase; DMAP1, DNMT1 Associated Protein-1; RFC, Replication factor complex; MeCP2, Methyl CpG-binding protein; HAT, Histone acetylase; HDAC, Histone deacetylase; HP1, Heterochromatin protein 1; ISWI, Imitation Switch protein; ORC, Origin Recognition Complex; MCM, Minichromosome maintenance protein; MYST, MOZ, Ybf2/Sas3, Sas2, and TIP60; Sas2, Something about silencing.

Introduction

In the course of each cell cycle, the genome is faithfully duplicated and divided between two daughter cells. In order to maintain the clonal inheritance of gene expression patterns, otherwise known as epigenetic inheritance, the cell must not only replicate its DNA, but also duplicate the structure of chromatin and its higher order packaging in the nucleus.[1] Following DNA replication, the DNA is methylated and packaged into nucleosomes by the binding of histone octamers to form chromatin.[2–4] The structure of chromatin may then be altered by chromatin-associated factors that modify the nucleosomes and thereby contribute to the formation of domains within the genome.[5] These modifications can render the chromatin more or less accessible to various nuclear processes including transcription and replication, participating directly in the formation of genetically active or inactive higher order chromatin structures, often referred to as euchromatin and heterochromatin, respectively. The mechanisms that maintain the complex structure of chromatin and the epigenetic state of the cell (chromatin replication) must act concurrently with DNA replication during S-phase.

Since the protein components of chromatin are rearranged and subsequently re-assembled during each round of replication, it seems reasonable to postulate that the events occurring during DNA replication play a key role in establishing and maintaining epigenetic states of chromatin. Furthermore, the replication of chromatin is temporally compartmentalized such that, with certain notable exceptions,[6,7] most heterochromatin is replicated late in S-phase while most genes are located within early replicating euchromatin (for recent reviews, see Refs. 8–11). Synthesizing certain types of chromatin regulators at specific times during the cell cycle or targeting them to replication forks at different times during S-phase would provide a convenient means to regulate chromatin structure at the level of replicon-sized chromosomal domains. Chromatin regulators may be recruited to replication forks by the replication proteins themselves. In *Saccharomyces cerevisiae*, mutations in several different replication protein genes affect epigenetic silencing but not DNA replication,[12,13] suggesting a role for replication proteins in establishing epigenetic states that is separable from their role in DNA synthesis. Several

recent studies have identified chromatin-modifying proteins that interact with components of the replication machinery and localize to sites of active DNA synthesis at specific times during S-phase. In this review, we summarize these findings and relate them to differences in chromatin assembly during early and late S-phase.

The mechanics of DNA replication and chromatin assembly

When cells are pulse-labeled with nucleotide analogs, DNA synthesis takes place at discrete sites within the nucleus, often referred to as replication foci (Fig. 1). Since euchromatin and heterochromatin occupy different compartments of the nucleus and replicate at different times, the distribution of replication foci is characteristic of the time during S-phase at which cells are labeled.[14–16] Early S-phase is distinguished by approximately 100–300 replication foci located throughout the nucleus and excluded from the nucleoli (Fig. 1). In late S-phase, replication foci are initially more prominent along the nuclear periphery and perinucleolar regions, and later at a few larger sites distributed throughout the nucleus (Fig. 1). Several proteins, involved in both DNA synthesis (e.g. DNA polymerases, PCNA, RFC, RPA, DNA ligase)[17] and epigenetic inheritance (e.g. DNMT1, CAF-1; discussed below),[18,19] have been localized to these foci throughout S-phase. Many of these proteins have also been shown to interact physically. In particular, the homotrimeric PCNA complex can interact with multiple proteins at the replication fork. For example, PCNA interacts with both CAF-1(chromatin-assembly factor-1) and DNMT1(DNA methyltransferase-1), providing a means to tether chromatin assembly proteins to the sites where DNA synthesis is taking place.[13,19–22] However, since they are associated with all sites of replication, these proteins are unlikely to be directly involved in establishing active versus inactive epigenetic states. Rather, the epigenetic state of the replicated chromatin is likely established by factors that interact with these proteins at specific times during S-phase.

The deposition of new histones throughout S-phase is controlled by histone chaperones, which associate with newly synthesized histones in the cytoplasm and accompany them into the nucleus and to sites of DNA synthesis.[3] The chromatin assembly factor 1(CAF-1) and the anti-silencing factor 1(Asf-1) protein bind tetramers of H3/H4.[3,23] The nucleosome assembly proteins Nap-1 and Nap-2 proteins bind dimers of H2A/H2B.[3,24] The histone chaperones provide a means of controling both the import of new histones as well as chromatin modifications following nucleosome assembly. For instance, Nap-2 is phosphorylated and excluded from the nucleus in G_1 and then dephosphorylated at the G_1–S boundary and imported into the nucleus, potentially carrying in newly synthesized histones H2A and H2B.[25] In addition, in *Drosophila* and human cells, Nap-1 associates with ACF, an ATP-dependent chromatin-remodeling complex that spaces nucleosomal arrays.[26,27] There is also evidence for

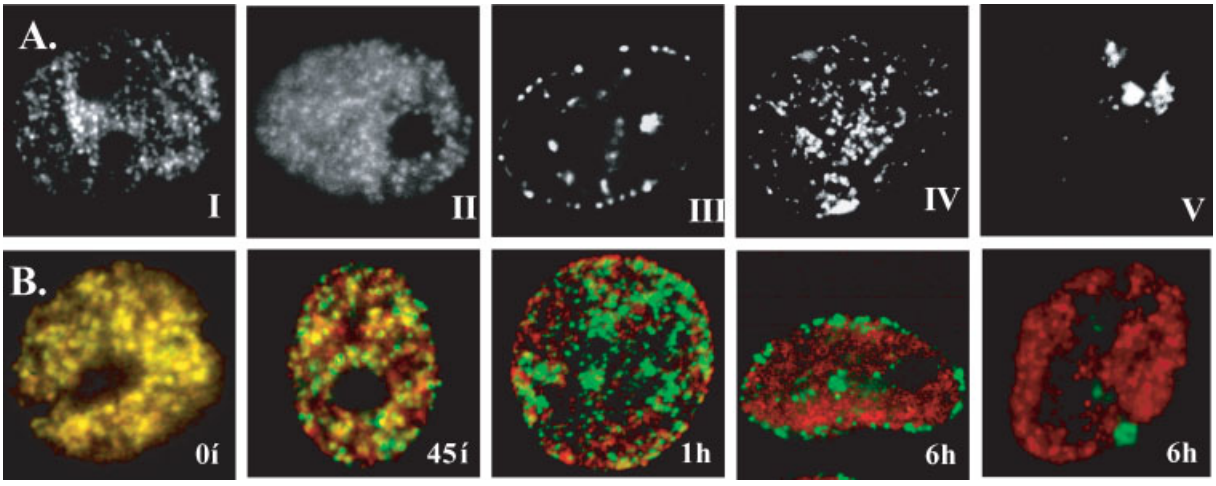

Figure 1. Visualizing differences in replication patterns in early versus late S-phase. Since euchromatic and heterochromatic chromosomal domains occupy specific sites in the nucleus and replicate at specific times, one can visualize characteristic patterns of replication when cells are labeled with nucleotide analogs at different times during S-phase. **A:** Cells were labeled with 5′-bromodeoxyuridine at different times during S-phase and the sites of DNA synthesis were visualized by immunofluorescence using anti-BrdU antibodies, enabling the detection of five specific replication patterns. **B:** The dynamics of replication are displayed by first labeling cells with chlorodeoxyuridine, chasing for the indicated period of time, and then labeling with iododeoxyuridine. This method allows one to distinguish early versus late-replication patterns during a single S-phase in asynchronous cells. (Adapted from Dimitrova and Gilbert, Molecular Cell 1999;4:983–993, with permission from Elsevier Science).

interactions between different chaperones, as in the case of CAF-1 and Asf-1, which when combined in an in vitro chromatin assembly reaction display synergistic activity.[28] The processes of nucleosome deposition and spacing, often referred to as chromatin maturation, occur concurrently with replication. Hence, the events taking place at the DNA replication fork provide a unique opportunity to modulate the structure of chromatin.

Euchromatin first

Early S-phase is associated with the replication of genetically active chromatin.[8] In fact, when sites of transcription are visualized by incorporation of bromouridine (BrU), a staining pattern similar to sites of early S-phase DNA synthesis is revealed, although transcription and replication do not occur at the same sites simultaneously.[29] A hallmark of transcriptionally active chromatin is the hyperacetylation of core histone tails. Acetylated histone H2A and H4 colocalize with CAF-1 at replication forks, but these acetates are rapidly removed from late- but not early-replicating chromatin.[30] H4 is post-translationally acetylated at lysines K5 and K12 by a cytoplasmic histone acetyltransferase (HAT), whereas H4 at early- but not late-replication foci is additionally acetylated at K8 and K16 (Fig. 2).[30,31] The additional acetylation at K8 and K16 presumably must come from a HAT that is specifically targeted to early- but not late-replicating sites.

In primary human cell lines and in *Drosophila*, there is evidence that the retinoblastoma protein (Rb) and members of the E2F family of transcription factors associate with replication foci.[32,33] Rb and E2F subunits were found to localize to sites of early- but not late-DNA synthesis in human fibroblasts.[32] In *Drosophila*, Rb and E2F-1 have been shown to interact with the origin recognition complex (ORC) to control DNA replication of the chorion genes.[33] Since Rb is a transcriptional repressor,[34] the localization of Rb near origins in both *Drosophila* and potentially in primary human cells may reflect its role in repressing the transcription of a specific subset of early replicating genes. Rb is capable of targeting HDAC1 and SUV39H1, a histone methyltransferase, to E2F regulated promoters.[35] HDAC-1 is bound to E2F promoters during G_1 and is released at the G_1–S transition to allow activation of transcription.[36] SUV39H1 methylates lysine K9 of histone H3, which then serves as a binding site for the heterochromatin chromatin protein, HP1.[37,38] K9-methylated H3 and HP1 are concentrated at late-replicating regions but have recently been shown to be present at specific early-replicating sites as well.[39] Hence, Rb recruitment of HP1 may represent a mechanism to silence early replicating genes that might otherwise be assembled into active chromatin. Although this is an intriguing model that deserves further consideration, the significance of Rb localization to sites of DNA synthesis in both human and *Drosophila* is not yet clear. The *Drosophila* studies have focused on events occurring at sites of uncontrolled gene amplification while the colocalization of Rb with sites of DNA synthesis in primary human cell lines has been challenged.[40]

Heterochromatin last

While there is little direct evidence for early S-phase-specific chromatin assembly activities, several proteins have been found to localize specifically to sites of late DNA replication (Fig. 3). It is not clear whether this is because more activities are required to assemble heterochromatin or whether early S-specific proteins have simply not been identified. It is possible that the consortium of chromatin regulators in early S-phase is a more complex group of proteins that do not localize generally to the population of early replicating foci. Alternatively, it may be particularly important for heterochromatin to be packaged in a consistent and recognizable form to facilitate the organization of the genome in a crowded nucleus. In this section, we will discuss recently identified chromatin modifications that take place at late-replication forks.

DNA methylation

In mammalian cells, DNA methylation generally serves as a marker for transcriptional repression.[41] The patterns of DNA methylation, primarily at sites of CpG, are maintained by the activity of DNMT1, which copies existing methylation patterns from the parental strand during DNA replication and repair.[41] During S-phase, DNMT1 localizes to both early and late-replication foci, along with an associated protein, DMAP1.[18] However, DNMT1 also interacts with proteins that are present specifically at late-replicating foci. For example, MBD2/3, a heterodimeric complex that binds methyl-CpG through its MBD2 subunit, is present specifically at late-replication foci and appears to recruit DNMT1 through its MBD3 subunit.[42,43] Interestingly, the MBD3 subunit also interacts with components of the ATP-dependent chromatin remodeling complex NuRD, which is associated with transcriptional repression, suggesting a means by which NuRD could be targeted to late S-phase replication forks, although there is as yet no direct evidence for this localization.[43] Significantly, in transiently transfected cells, DNMT1 interacts with HDAC2 at late- but not early-replication foci.[18] The presence of HDAC2 at late-replication foci provides a means to mark methylated DNA as transcriptionally silent at the nucleosomal level through the deacetylation of core histones.

In contrast to DNMT1, the two other major mammalian DNA methyltransferases, DNMT3a and DNMT3b, can facilitate de novo DNA methylation and exhibit different localizations during the cell cycle.[44] In mouse embryonic fibroblasts, DNMT3a colocalizes with HP1 and the methyl-DNA-binding protein MeCP2 at late-replicating pericentromeric heterochromatin throughout the cell cycle, whereas DNMT3b staining remains diffusely nuclear.[44] In contrast, in embryonic stem cells, DNMT3b colocalizes at pericentromeric sites with

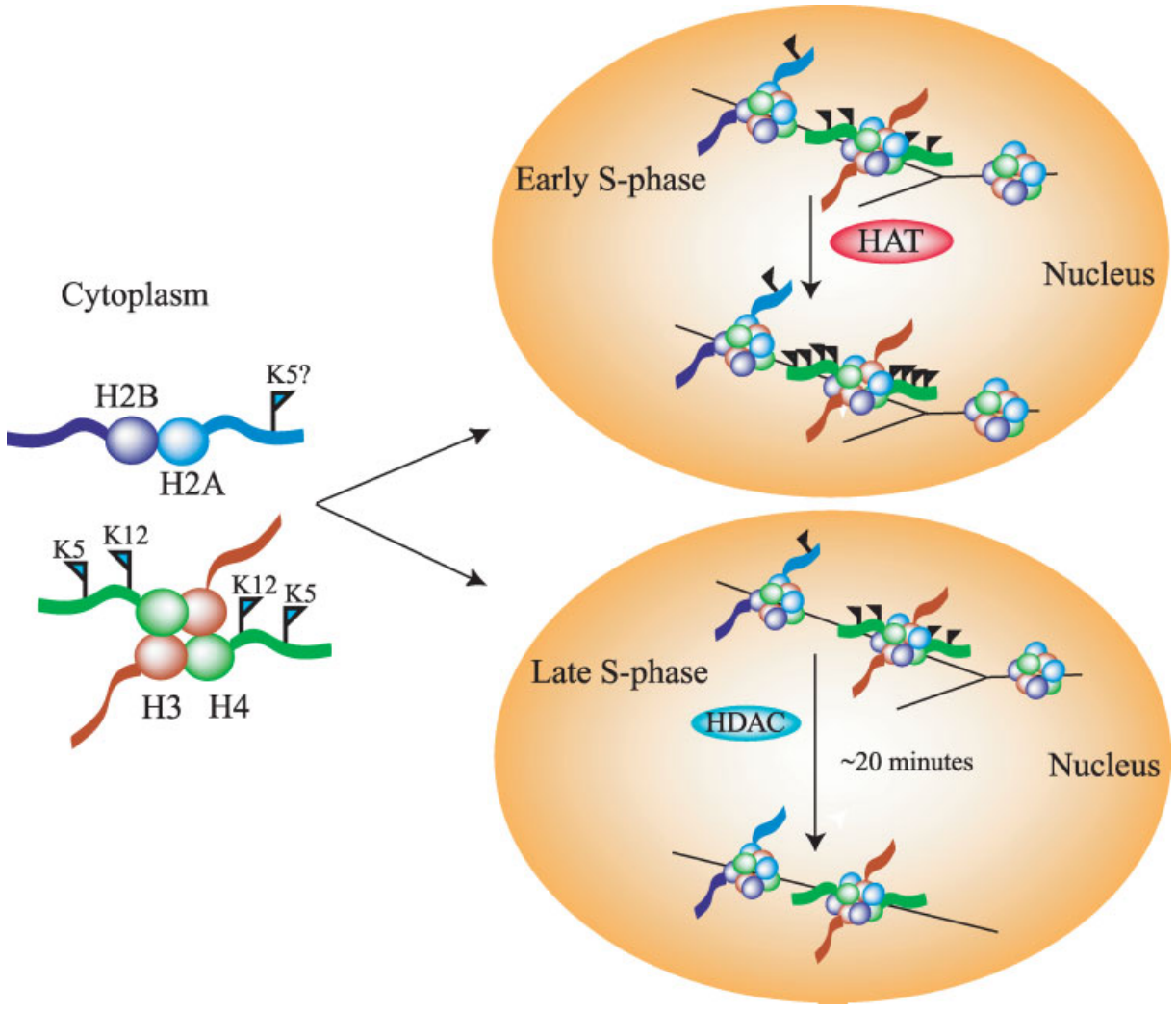

Figure 2. Differential acetylation states of histones in early versus late S-phase and the semi-conservative nature of nucleosome assembly. All newly synthesized histone H4 (green) is acetylated (black flags) at lysines 5 and 12 by a cytoplasmic HAT. Similarly, H2A (blue) is acetylated on lysine 5 in early S-phase, although it is not known whether this acetylation takes place in the cytoplasm or the nucleus. In mammals, newly synthesized H3 (red) is not acetylated prior to assembly into nucleosomes, although it is in some species.[98] Similarly, there is currently no evidence for the acetylation of newly synthesized H2B (purple) in the cytoplasm. Newly synthesized H3 and H4 form tetramers and H2A forms dimers with H2B in the cytoplasm. Inside the nucleus, newly synthesized H3–H4 tetramers deposit with parental H2A–H2B dimers, and newly synthesized H2A–H2B dimers deposit with parental H3–H4 tetramers. In early S-phase, H4 becomes additionally acetylated at lysines 8 and 16 due to the activity of an unidentified nuclear HAT. All of these acetates remain on early replicating chromatin throughout most of the cell cycle. In contrast, during late S-phase acetates on H4 lysines 5 and 12, as well as lysine K5 of H2A are rapidly lost following incorporation into chromatin, likely due to the presence of an HDAC at the site of chromatin assembly. HDAC2 is known to specifically localize to sites of late DNA synthesis[18] (see Fig. 3).

DNMT3a and HP1.[44] Hence, the change in localization of these de novo DNA methyltransferases at an early developmental stage may provide a mechanism to establish an epigenetic state that is thereafter maintained by DNMT1.

Histone acetylation

Chromatin assembly at late S-phase replication forks is mediated by the same histone chaperones as in early S-phase. However, there is a change in the acetylation of incorporated histones in late S-phase that is not observed during early replication. Although histone H4 is acetylated at lysines 5 and 12 when it enters the nucleus, and accumulates at late-replicating foci, these acetates are removed approximately 20 minutes after DNA synthesis is completed.[30,31] Removal of these acetates can be prevented by treating cells with TSA, an HDAC inhibitor,[30] demonstrating that this HDAC activity

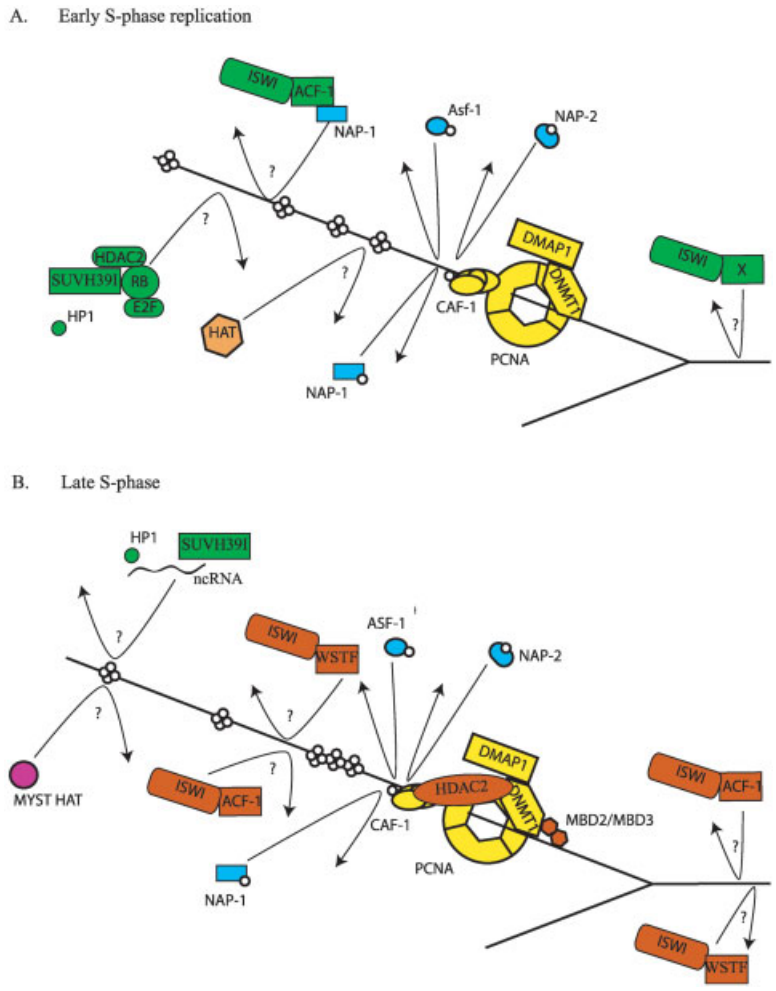

Figure 3. Chromatin modifiers at replication forks. Proteins present at replication forks in both early and late S-phase are shown in yellow. Proteins specific to late S-phase appear in red. Histone chaperone proteins are shown in light blue and the small white circles represent histones. Proteins in green represent those proteins that are likely to facilitate higher order chromatin structure but have yet to be specifically localized to the replication fork. Arrows indicate the transient association of chromatin modifiers with the newly assembled chromatin. See text for additional details.

must be in close proximity to replication forks. In fact, this as yet unidentified HDAC must be localized specifically to late-replication forks because H4 K5,12 acetates are not removed from the earlier-replicated chromatin. In addition, H4 K8,16 acetates persist at early-replicating sites throughout the cell cycle but never appear at late-replicating sites, even after TSA treatment,[30] implying that the HAT activity responsible for H4 K8,16 acetylation is excluded from late-replicating sites even though it is in the nucleus during late S-phase to maintain these modifications on euchromatin. These findings demonstrate the differential localization of HATs and HDACs to replication forks at different times during S-phase (Fig. 2).

Histone acetylation is typically associated with genetically active chromatin, however, evidence exists that acetylation also functions in gene silencing and replication origin activation. In both *S. cerevisiae* and *Drosophila*, MYST domain HATs have been implicated in gene silencing.[45–47] The MYST domain HATs are conserved from yeast to humans and appear to have roles in transcriptional silencing. In *S. cerevisiae*, the Sas2 protein has been identified as a MYST-domain HAT that acetylates H4 at lysine 16.[46,48] This acetylation event is essential for the silencing at HML, but has opposite effects on HMR, ribosomal genes, and telomeres,[46,48–50] suggesting that other chromatin modifiers such as the Sir proteins play a role in these different types of silent chromatin in yeast.[51] Sas2 genetically interacts with the ORC1 subunit of ORC and physically interacts with components of chromatin assembly factors such as the CAC1 subunit of CAF-1 and Asf-1.[46,48,52] In *Drosophila*, the recently identified Chameau protein is also a MYST domain HAT that is required for transcriptional repression.[47] Chameau is the homologue of a human MYST-domain HAT, HBO1, which interacts with the pre-replication complex proteins ORC1 and MCM2.[53,54] The exact residues acetylated by Chameau and HBO1 have yet to be identified, however, there is evidence

that acetylation of H4 K12 may be a marker of heterochromatin in animal cells, similar to acetylation of H4 K16 in *S. cerevisiae*.[45,46,48]

Targeting histone modifications to nucleosomes near ORC1 and MCM2, which are bound to replication origins, could provide a means to regulate the time of origin firing, dictating the very replication timing program that is in turn necessary for inheritance of these modifications. Direct evidence for such a relationship comes from several recent studies in *S. cerevisiae*.[55–58] Deletion of silent chromatin proteins Sir3 and Ku advances the replication timing of certain telomeric origins,[56,57] deletion of the Rpd3 HDAC advances the replication timing of several internal late-replicating origins,[58] while deletion of the Sir2 HDAC increases the frequency of origin firing within rDNA.[55] In addition, targeting of the silent chromatin protein Sir4[59] or the Gcn5 HAT[58] to a specific origin causes a delay or advancement, respectively, of its replication timing. In mammalian cells as well, HDAC inhibitors can advance the replication timing of at least some chromosomal sequences.[60] Thus, the level of histone acetylation in or around the replication origin can directly influence the timing of origin firing.

Chromatin remodeling

Chromatin remodeling complexes are large multiprotein complexes that have been identified as transcriptional regulators in a number of organisms and use ATP-hydrolysis and/or histone deacetlyation to catalyze changes in chromatin structure.[61] Recently, two chromatin remodeling complexes, WICH (for WSTF-ISWI chromatin remodeling complex) and ACF, have been localized specifically to sites of late- but not early-replication in mammalian cells.[62,63] Both complexes contain the ISWI protein, which is homologous to the Swi2/Snf2 DNA-dependent ATPase subunit of the *S. cerevisiae* Swi/Snf chromatin remodeling complex and is a component of many remodeling complexes.[61] The WSTF subunit of WICH is nearly identical in its subdomain architecture to the ACF-1 subunit of ACF.[62,64] WSTF and ACF-1 exhibit similar localization during the cell cycle except during mitosis, when WSTF, but not ACF-1, localizes to condensed chromosomes.[62,63] During G_1 and early S-phase, WSTF and ACF-1 are diffusely nuclear and excluded from the nucleolus.[62,63] Coincident with the onset of replication at pericentromeric sites in late S-phase, both proteins are present in large foci that colocalize with HP1 at these sites.[62,63] Depletion of ACF by siRNA in human cells results in a decrease in the rate of DNA synthesis specifically during late S-phase.[63] In contrast, depletion of ISWI subunit of these complexes by siRNA causes a generalized decrease in the rate of DNA synthesis throughout S-phase.[63]

These results can be interpreted in two ways, either or both of which may be correct. The S-phase delay when either ACF or ISWI is depleted could be due to a requirement for these remodeling complexes ahead of late-replication forks in order to open condensed chromatin and efficiently replicate DNA. However, an alternative interpretation is that their primary role is to assemble chromatin after replication, and that defects in replication-coupled chromatin remodeling activate a checkpoint response that stalls DNA synthesis. In *Xenopus* egg extracts, which lack many checkpoint controls, immunodepletion of WSTF, ACF, or ISWI does not effect replication, but does affect the proper spacing of nucleosomes along the chromatin, with depletion of WSTF being the most severe.[65] This implies that these complexes are not required for DNA replication per se. However, in this system, a role in opening heterochromatin prior to replication may be masked by the fact that condensed heterochromatin is not assembled in *Xenopus* egg extracts.[66] In summary, these new findings provide direct evidence for two different chromatin remodeling complexes that are targeted to replication forks specifically during late S-phase, although their precise roles remain to be determined.

Histone methylation and HP1 binding

The HP1 proteins are highly conserved heterochromatin proteins that have been demonstrated to affect position effect variegation, a form of transcriptional silencing, in both *Drosophila* and mammals.[67,68] HP1 proteins appear to be essential for cell viability as inhibition of HP1 function by the expression of intracellular antibodies leads to cell death in mammalian cells.[69] Recently, the amino terminal tail of histone H3, when methylated at K9, was determined to be a binding site for HP1.[37] The methyltransferase involved was identified as SUV39H1 in both *Drosophila* and mammals.[30,70,71] SUV39H1 localizes to pericentromeric heterochromatin along with HP1 isoforms in both *Drosophila* and mammalian cells, although the colocalization is not complete.[72,73] Mammals have two redundant SUV39H1 proteins, SUV39H1 and SUV39H2, and the absence of both SUV39H1 and SUV39H2 in double knock-out mouse cells reduces the binding of HP1 to heterochromatin.[70,72] However, the methyl-K9-H3 modification is not responsible for all HP1 chromatin binding, as there are chromosomal regions that are methyl-K9 H3 positive, but lack HP1 and vice versa.[74] Moreover, as the methyl-K9-H3 modification persists throughout the cell cycle,[74] it remains to be determined if the activity or localization of SUV39H1, or any other histone methyltransferase, is cell-cycle regulated.

There are likely to be additional factors involved in targeting SUV39H1 and HP1 to heterochromatic sites. Along these lines, a new binding partner of HP1, heterochromatin protein 2 (HP2), was recently identified in *Drosophila*.[75] HP2 is a large protein present in two isoforms of 176 and 356 kDa. Both function as suppressors of position effect variegation and colocalize with HP1 on polytene chromosomes. The larger isoform is an HMG-motif protein, containing two AT-hooks that

can bind AT-rich DNA and induce conformational changes in the structure of DNA.[75,76] Both isoforms of HP2 also contain a chromodomain, found in several chromatin proteins and known to mediate the assembly of higher order chromatin structures.[68] It will be interesting to determine whether HP2 proteins are localized to replication foci at different times during S-phase.

Non-coding RNA

Intriguingly, the chromodomains of some proteins have been shown to bind RNA, and recent evidence points to a role for RNA in mediating heterochromatin formation.[77,78] Treatment of mammalian cells with RNase results in the loss of HP1 staining from pericentromeric regions.[78] Therefore, the association of HP1 proteins with methyl-K9-H3 may be enhanced by an unidentified RNA component. The HP1 proteins consist of an N-terminal chromodomain and a C-terminal chromoshadow domain separated by a hinge region.[68] The hinge region of HP1 has been found to exhibit RNA-binding activity in gel-shift assays and HP1 binding to heterochromatin requires both an intact chromodomain and an RNA component.[79] In addition, recent work in *S. pombe* has uncovered a link between gene silencing by siRNA and chromodomain-containing proteins such as Clr4, the *S. pombe* homologue of SUV39H1, and Swi6, the homologue of HP1.[80] The proposed model is that Clr4 binds to the short dsRNA and is targeted to specific genes, where it methylates the histones and recruits Swi6 to silence the gene.[80] The conclusion from these experiments is that the formation of siRNA and the interaction of chromatin proteins with RNA may be central to the regulation of higher order chromatin structure.

Although there is as yet no evidence for a role of these RNA components in the maturation of chromatin at replication forks, an intriguing liaison between non-coding RNA and late replication clearly exists in the case of mammalian X-chromosome inactivation. Accumulation of a non-coding RNA, Xist, on one of the two X chromosomes precedes a switch from early to late replication and transcriptional inactivation of this chromosome.[81] Genetic inactivation of the X-chromosome is reversible until the switch in replication timing, which takes place within one cell cycle of Xist accumulation and is one of the first of a series of epigenetic modifications of the inactive X.[81] A role for non-coding RNA in heritable epigenetic states has also been proposed for imprinted genes such as IPW (imprinted in Prader-Willi syndrome) and H19 (imprinted maternally expressed untranslated mRNA), the alleles of which also begin to replicate asynchronously early in development, similar to X-chromosome inactivation.[82–85] Hence, the potential role of non-coding RNA and RNA-binding proteins in the propagation of heritable chromatin states during S-phase will be an important area of investigation.

Copying the histone code

The escalating array of histone modifications and chromatin modifiers predicts that there is an enormous complexity to the different types of chromatin that can be assembled, with each type possibly having a unique meaning in the context of a histone code.[86,87] Recent whole-genome studies using microarray technology have revealed that subdomains of chromatin may be differentiated based upon their regulation by a specific histone modifier. For example, in *S. cerevisiae*, the genes affected by a deletion of the HDAC Hda1 were clustered in the subtelomeric regions, termed HAST domains (Hda1-affected subtelomeric region).[88] In mice, knockout of the G9a histone methyltransferase affected the expression of a specific class of genes.[89] In *Drosophila*, a significant portion of the genome is organized into blocks of chromatin-containing groups of functionally unrelated genes that have similar levels of transcriptional activity.[90] As transcriptional activity is determined by histone modifications, it will be interesting to determine whether these blocks of similarly expressed genes represent chromatin domains with characteristic histone modifications.[86,87] Compartmentalizing these domains into units that are replicated at specific times during S-phase would provide a convenient means to ensure the fidelity of their inheritance.

How can the language of histone modifications be passed on to subsequent generations? One answer may lie in the mechanism by which nucleosomes are inherited (Fig. 2). During S-phase, nucleosomes are dissociated into H3–H4 tetramers and H2A–H2B dimers[91] but the acetylation state of these histones does not appear to be affected.[92] After DNA synthesis, newly synthesized H3–H4 tetramers assemble with pre-existing H2A–H2B dimers, whereas newly synthesized H2A–H2B dimers assemble with pre-existing H3–H4 tetramers.[93,94] This semiconservative distribution of new and old histones provides a potential means for the inheritance of epigenetic states, as it ensures that each and every newly assembled nucleosome contains a set of subunits with the pre-existing modifications, which could in turn facilitate restoration of the complete set of original modifications. There is some evidence for crosstalk between subunits within nucleosomes. Ubiquitination of H2B directs the methylation of lysine 79 of histone H3, one marker of transcriptionally active chromatin.[95,96] Hence, just as the sequence of bases in each parental DNA strand ensures inheritance of the genome by dictating the sequence of bases in the nascent strand, the set of modifications in parental histones could ensure epigenomic inheritance by dictating the set of modifications on nascent histones.

Conclusions and future directions

Recent evidence has demonstrated a link between DNA replication and epigenetic inheritance that is mediated by the interaction of chromatin modifiers with components of the

replication machinery. In early S-phase, the duplication of the epigenome is shifted towards the replication of actively transcribed regions, whereas in late S-phase, the chromatin modification machinery shifts towards the inheritance of silent chromatin and the re-establishment of heterochromatin domains. This shift may represent a global change in the environment inside the nucleus as a recent study has found that reporter plasmids microinjected into early S-phase cells are assembled into transcriptionally active, hyperacetylated, chromatin while those microinjected late in S-phase are assembled into transcriptionally inactive, hypoacetylated, chromatin.[97] These studies underscore the importance of replication timing in maintaining patterns of gene expression. Identifying the gene products that modify chromatin during replication and those that regulate the replication timing program during S-phase will be critical for our understanding of how epigenetic chromatin states are maintained and how they can be reversed in order to re-program nuclei to the totipotent state.

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