GENETICS

Replicating nucleosomes

Srinivas Ramachandran[1,2] and Steven Henikoff[1,2]*

10.1126/sciadv.1500587

Eukaryotic replication disrupts each nucleosome as the fork passes, followed by reassembly of disrupted nucleosomes and incorporation of newly synthesized histones into nucleosomes in the daughter genomes. In this review, we examine this process of replication-coupled nucleosome assembly to understand how characteristic steady-state nucleosome landscapes are attained. Recent studies have begun to elucidate mechanisms involved in histone transfer during replication and maturation of the nucleosome landscape after disruption by replication. A fuller understanding of replication-coupled nucleosome assembly will be needed to explain how epigenetic information is replicated at every cell division.

INTRODUCTION

Animals and plants develop from a single-celled zygote to a multicellular organism with scores of different cell types that perform a multitude of unique functions and yet carry the same genome. This diversity in cellular identity during development and adulthood is achieved by differential gene activation and repression in distinct cell types. Gene expression is influenced by nucleosomes, which mediate accessibility and hence all biochemical activities on the genome. The overall inhibitory role of nucleosomes on genomic access is modulated by posttranslational modifications of histones in the nucleosome and the replacement of canonical histones in the nucleosome by histone variants. Thus, the pattern of differential gene expression that contributes to cellular identity is reflected in features that differentiate nucleosomes along the genome, the "nucleosome landscape." The major histone modifications classically associated with repressed chromatin domains include H3K9 trimethylation (H3K9me3), which is found in constitutive heterochromatin, and H3K27 trimethylation (H3K27me3), which is found in facultative heterochromatin. The major histone variants include H3.3, H2A.Z, and the centromeric H3 variant, cenH3. In metazoans, H3.3 and H2A.Z are broadly distributed throughout the genome, but are differentially enriched at particular regions including actively transcribed regions, whereas cenH3 is rarely found outside of centromeres.

The nucleosome landscape of different cell types is established in large part through processes that disrupt and replace nucleosomes, mainly transcription, chromatin remodeling, and replication. Examples of transcription and remodeling establishing the cell type–specific nucleosome landscape include widespread transcription at enhancers (*1*), remodeling (*2*), and nucleosome turnover (*3*) at Polycomb response elements (PREs) in *Drosophila*, which are sites required for maintaining cell type–specific transcriptional programs through several cell divisions during development.

For eukaryotic development to proceed, the nucleosome landscape must be reproduced by default in daughter cells after DNA replication, which proceeds bidirectionally from origins bound by the origin recognition complex. The replication fork consists minimally of the minichromosome maintenance (MCM) helicase and DNA polymerase, both of which are loaded at replication origins at the beginning of replication, and the replicative processivity clamp, PCNA. The MCM complex forms a channel around a single strand of DNA (the leading strand of replication) and unwinds DNA in front of it (*4*). An MCM channel translocating on single-stranded DNA will result in the complete disruption of the nucleosome. Thus, over a few seconds of passage of the helicase and the replication machinery behind, there is massive disruption that dissociates the prereplication nucleosome landscape.

After the new strand of DNA is synthesized by the polymerase, assembly factors reconstitute old nucleosomes and assemble new nucleosomes behind the replication fork. Because there is a doubling of genetic material during replication, cells require double the amount of the genome packing material, namely, the histones. To provide sufficient material at S phase, there is massive synthesis of the four canonical histones, which form the nucleosome core that wraps 147 base pairs (bp) of DNA, and the linker histone H1, which cements an additional ~20-bp DNA wrap to the nucleosome surface. Newly replicated chromatin must incorporate both the preexisting histones disrupted by the replication fork and the newly synthesized histones. The main questions for elucidating the inheritance of nucleosome features during replication are as follows: How are old and new histones incorporated during replication-coupled nucleosome assembly? How is the nucleosome landscape altered during replication?

Stable inheritance of epigenetic information through replication is exemplified by cytosine methylation at CG dinucleotides in most animals and plants. At CG dinucleotides, methylation is symmetric, that is, the cytosine on both strands is methylated. At replication, each daughter CG dinucleotide inherits one of the two symmetrically methylated cytosines from the parental strand, base-paired to an unmethylated cytosine on the newly synthesized strand, that is, a hemimethylated CG dinucleotide. The Dnmt1 methyltransferase recognizes the hemimethylated CG dinucleotide and methylates the cytosine on the newly synthesized strand. Thus, the methylation status of parental CG dinucleotides is inherited by the daughter chromosomes through the hemimethylated state. In this review, we describe examples where nucleosomes are "replicated" on the daughter chromosomes analogous to cytosine methylation at CG dinucleotides, with the goal of delineating the underlying mechanisms.

NUCLEOSOME INHERITANCE THROUGH REPLICATION

To understand how nucleosomes are inherited through replication, we need to first consider the constraints posed by the structure of the nucleosome. The nucleosome core consists of the centrally located H3-H4 tetramer flanked on either side by an H2A-H2B dimer. Nucleosomal DNA enters at the "proximal" H2A-H2B dimer and exits at the "distal"

[1]Howard Hughes Medical Institute, Seattle, WA 98109, USA. [2]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.
*Corresponding author. E-mail: steveh@fhcrc.org

H2A-H2B dimer. The twofold mirror symmetry of the nucleosome means that half the length of nucleosomal DNA (73 bp) wraps one-half of the histone octamer consisting of one each of the H2A-H2B and H3-H4 dimers. DNA-histone contacts at the entry and exit sites of the nucleosome are the most labile, and these contacts can be lost during normal thermal fluctuations, resulting in transient unwrapping of nucleosomes (*5*). This results in H2A-H2B dimers being more susceptible to loss and replacement than H3-H4. In contrast, the central location of the H3-H4 tetramer is expected to restrain its turnover, which in experimental measurements is markedly lower than that of H2A-H2B (*6*). Tracking nucleosome inheritance then becomes primarily a problem of tracking the H3-H4 tetramer through replication.

The nucleosome landscape as defined by the identity of H3 and H4 would be marked by H3 variants H3.1/H3.2 or H3.3 and posttranslational modifications on H3 and H4. Posttranslational modifications associated with active chromatin are predominantly found on H3.3 compared to H3.1/H3.2, whereas modifications that are part of the machinery involved in repression (H3K27me and H3K9me) are predominantly found on H3.1/H3.2 (*7*). Inheritance of a silenced region through replication would in some scenarios involve inheritance of H3-bearing modifications associated with repression at the same genomic location. Alternatively, the silenced region could be set up afresh each time after replication. Of the several modes of nucleosome assembly, replication-coupled assembly is distinguished by the fact that the canonical H3 (H3.1 and H3.2, hereinafter referred to as H3) is incorporated into chromatin only through this pathway (*8*). Replication-independent pathways of nucleosome assembly strictly incorporate only H3.3 (*8*).

Given alternative pathways and mechanisms to maintain the nucleosome landscape, we need to determine what is the relative significance of replication-coupled assembly in maintaining the nucleosome landscape through inheritance. Whereas histones are essential, disruption of replication-coupled assembly is not lethal in the budding yeast, *Saccharomyces cerevisiae*, indicating that nucleosome assembly pathways are largely redundant in this organism. However, disruption of replication-coupled assembly results in derepression of silent loci (*9*–*12*). A requirement for replication-coupled assembly to maintain silencing is also seen in the worm, *Caenorhabditis elegans*, where deletion of 11 residues in the H3 C terminus in 1 of 24 H3 genes results in the loss of asymmetry in the identities of a neuron pair (*13*). Deletion of these residues in H3 is predicted to destabilize the nucleosome due to loss of contacts that stabilize the H3-H4 tetramer interface. The H3 C-terminal deletion is phenocopied by disruption of replication-coupled assembly, which suggests that impaired replication-coupled assembly can affect developmental decisions. Here, similar to the situation in budding yeast, inefficient assembly or assembly of destabilized nucleosomes (with mutant H3) results in replication-independent assembly pathways filling in the "holes" in the nucleosome landscape with H3.3 after replication. The replication-coupled assembly pathway is also essential for development in plants (*Arabidopsis thaliana*) (*14*, *15*) and in flies (*Drosophila melanogaster*) (*16*, *17*), even in the presence of alternate nucleosome assembly pathways. In cultured human cells, disruption of replication-coupled assembly results in increased replication-independent incorporation of H3.3 (*18*). Because old H3.3 is enriched in histone modifications that are primarily associated with active chromatin, it is possible that spurious incorporation of H3.3 could disrupt silencing at inactive regions.

The importance of replication-coupled nucleosome assembly in maintaining silent chromatin states in yeast and for proper development in worms, flies, and plants suggests a role for this pathway in epigenetic inheritance. Direct evidence for the essentiality of the maintenance of histone variants through replication is exemplified by cenH3, the histone variant that replaces H3 at centromeres. Loss of cenH3 maintenance through replication can result in loss of centromere identity (*19*).

TRACKING CHROMATIN INHERITANCE THROUGH REPLICATION

Nucleosome inheritance would involve not only the transfer of old histone variants and posttranslational modifications to the newly synthesized chromosomes but also preservation of the genomic location of the old histones. In principle, we can track nucleosome inheritance at multiple levels: in bulk, at the level of genomes or chromosomes, at the level of large chromatin domains (~10 kb), and at the single nucleosome level. Currently, we have solid evidence for chromatin inheritance only at the bulk level.

Several methods have been developed recently to temporally track proteins associated with replication forks by isolating newly replicated chromatin labeled with nucleotide analogs (*20*–*22*). Combining the isolation of newly replicated chromatin with differential labeling of old and new histones with stable isotopes has enabled the quantification of the extent of transfer of old, modified histones and histone variants at the replication fork (*23*). This method also has high temporal resolution, enabling pulse-chase experiments to track the maturation of newly assembled chromatin over time. In cultured human cells, this method showed that only a small fraction of old H2A.Z is retained in the newly replicated chromatin and that H2A.Z is incorporated much later than fork progression, indicating little or no inheritance of H2A.Z during replication. H3.3 levels in newly replicated chromatin are the same as in bulk chromatin, indicating fast incorporation of both old and new H3.3 into the newly replicated chromatin. Thus, with respect to histone variants, H3.3 levels drop twofold immediately after replication, indicative of random segregation to both daughter chromosomes, whereas H2A.Z levels drop much more, indicating loss of H2A.Z in the daughter chromosomes. With respect to histone modifications, the newly replicated chromatin has half the levels of H3K27me3 and H3K9me3 compared to total H3. This could be attributed to equal distribution of old H3K27me3 and H3K9me3 between the daughter chromosomes, as evidenced by the lack of these methylation marks in newly synthesized H3.

H3K27me3 is deposited by the Polycomb repressive complex 2 (PRC2) (*24*, *25*). H3K27me3 is part of the machinery that maintains transcriptional repression of large chromatin domains over successive cell divisions (*26*). In the embryonic germ line of worm hermaphrodites, H3K27me3 is maintained by PRC2 on maternal chromosomes but remains absent from previously unmodified paternal chromosomes (*27*). Conversely, in a PRC2 null embryo, H3K27me3 containing paternal chromosomes retain the modified nucleosomes during twofold dilution at each cell division. This provides direct evidence of posttranslationally modified histone inheritance through successive cell divisions (*27*).

Spatially restricted inheritance of old histones has also been observed in germline stem cells in fly testes (*28*). These stem cells are attached to a cellular structure called the hub, and the daughter cells that go on to differentiate can be clearly distinguished from the stem cells spatially. When the fates of old and newly synthesized histones were

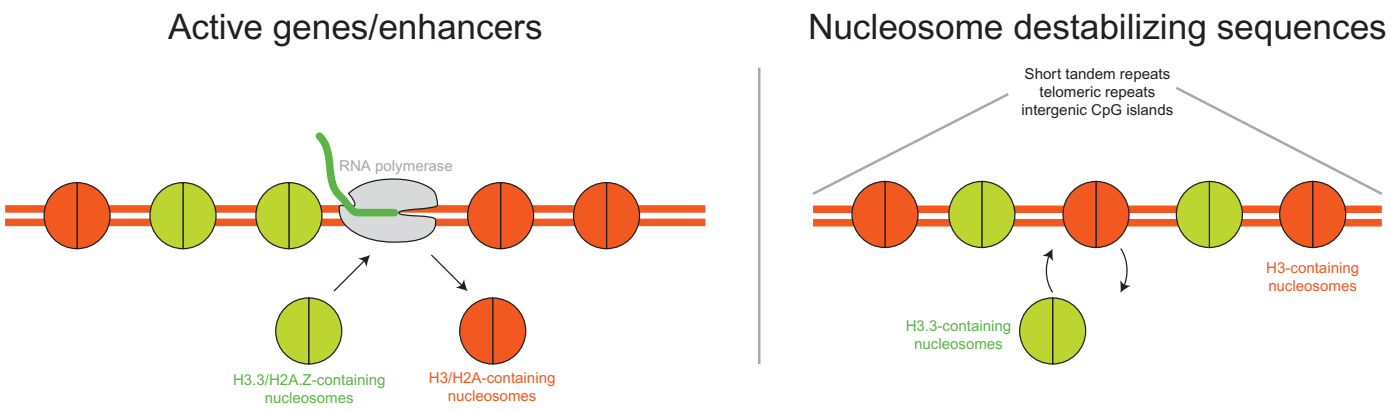

Fig. 1. Distinct mechanisms of incorporation of H3.3 at active and silent regions of the genome. At transcriptionally active regions, nucleosomes are disrupted by RNA polymerase or nucleosome remodelers, resulting in occasional nucleosome loss, with replacement by nucleosomes containing H3.3, which unlike H3 is present throughout the cell cycle. At regions of unusual base composition, which include telomeres, CpG islands, and short-period satellite repeats, the lack of nucleosome-stabilizing sequences results in relatively frequent nucleosome loss, with replacement by H3.3 nucleosomes.

traced over mitosis, there was a striking observation that old histones are preferentially retained in the chromosomes that segregated to the stem cell. Similarly, histones newly synthesized during the most recent S phase are preferentially retained in the chromosomes that segregate to the differentiating daughter cell. Thus, retention of old histones correlates with stem cell identity in the fly testes.

In summary, in ordinary cell divisions, there is equal distribution of old H3, H3.3, H3K27me3, and H3K9me3 to daughter chromosomes. Newly synthesized H3 lacks these methyls when deposited, resulting in a dilution of cellular levels of methylated H3 right after replication (*23*). At least in the case of H3K27me3, this distribution to daughter chromosomes occurs locally in worm embryos, thus preserving the genomic location of the old H3K27me3 (*27*). Similarly for cenH3, genomic location is preserved after replication in multiple organisms, and the total levels are diluted to one-half after replication (*29*). In budding yeast, there is no inheritance of cenH3, because the single cenH3 nucleosome at each centromere is lost during replication (*30*).

MECHANISMS FOR INHERITING NUCLEOSOME STATES THROUGH REPLICATION

Different genomic regions feature distinct mechanisms to maintain chromatin states after nucleosome disruption and dilution due to replication. Transcriptionally active regions are enriched for nucleosomes containing H3.3 and H2A.Z, both of which are exclusively incorporated by replication-independent nucleosome assembly pathways. H3.3 incorporation mirrors nucleosome turnover (*3*), presumably because, outside of replication, canonical H3 is not synthesized, and so H3.3 is the only substrate available to replace the H3 that is lost. H3.3-specific chaperones include DAXX (*31*, *32*) and HIRA (*33*), both of which selectively bind H3.3 over canonical H3. Thus, transcriptionally active regions and other sites that feature elevated rates of nucleosome turnover would accumulate H3.3 (*34*). Accumulation of H2A.Z at promoters and gene bodies has been proposed to be driven by disruption of H2A-H2B dimers from the nucleosome during transcription (*35*). Similarly, enhancers feature high nucleosome remodeler activity (*36*) and transcription (*1*), which is also reflected by high nucleosome turnover (*37*), explaining the enrichment of H3.3 and H2A.Z at these sites. Transcriptionally silent regions of unusual base composition that lack nucleosome-stabilizing features, such as short period tandem repeats, telomeric repeats, and intergenic CpG islands, also show evidence of high nucleosome turnover (*38*–*41*). Thus, the context of H3.3 incorporation dictates whether H3.3 is associated with active or silent chromatin states (Fig. 1). In summary, events outside of replication can overcome dilution of H3.3 and H2A.Z during replication-coupled assembly and dynamically maintain H3.3 and H2A.Z landscapes.

Histone modifications are diluted by half during replication. Hence, it is interesting to determine whether the modified histone levels immediately before replication or immediately after replication are required for their biological function. The replenishment of H3K27me3 and H3K9me3 landscapes is a slow process, completing only before the next round of replication, which indicates that lower postreplication levels are enough for the biological function of these modified histones (*23*). This suggests that methylation of newly synthesized histones before the subsequent S phase exists primarily to prevent further dilution of these landscapes over the next round of replication rather than to maintain silencing in the absence of replication. In vitro (*42*) and in vivo (*43*), evidence suggest that the diluted, old H3K27me3 can target methylating machinery to nearby nucleosomes containing newly synthesized histones. The PRC2 complex includes a catalytic subunit and an EED subunit (Esc in fly). Structural and biochemical characterizations of EED have revealed its ability to bind H3K27me3, a modification that is catalyzed by the EZH2 subunit of the PRC2 complex (*42*). When EED binds H3K27me3, the enzymatic activity of EZH2 increases sevenfold in vitro (*42*), leading to a template-binding model (Fig. 2) where binding of EED to old H3K27me3 could stimulate the activity of EZH2 on nucleosomes with newly synthesized histones that are not yet methylated. This model requires faithful inheritance of old histones on daughter chromosomes close to their original location on the parent chromosome and is yet to be directly demonstrated in vivo. A subunit of PRC2 in the budding yeast *Cryptococcus neoformans*, Ccc1, also binds H3K27me3 (*43*). The specific binding of Ccc1 to H3K27me3 is required for the maintenance of H3K27me3 and silencing of genes at subtelomeric regions. The action of Ccc1 might be explained in either of two ways: Ccc1 binding old H3K27me3 could direct PRC2 to methylate

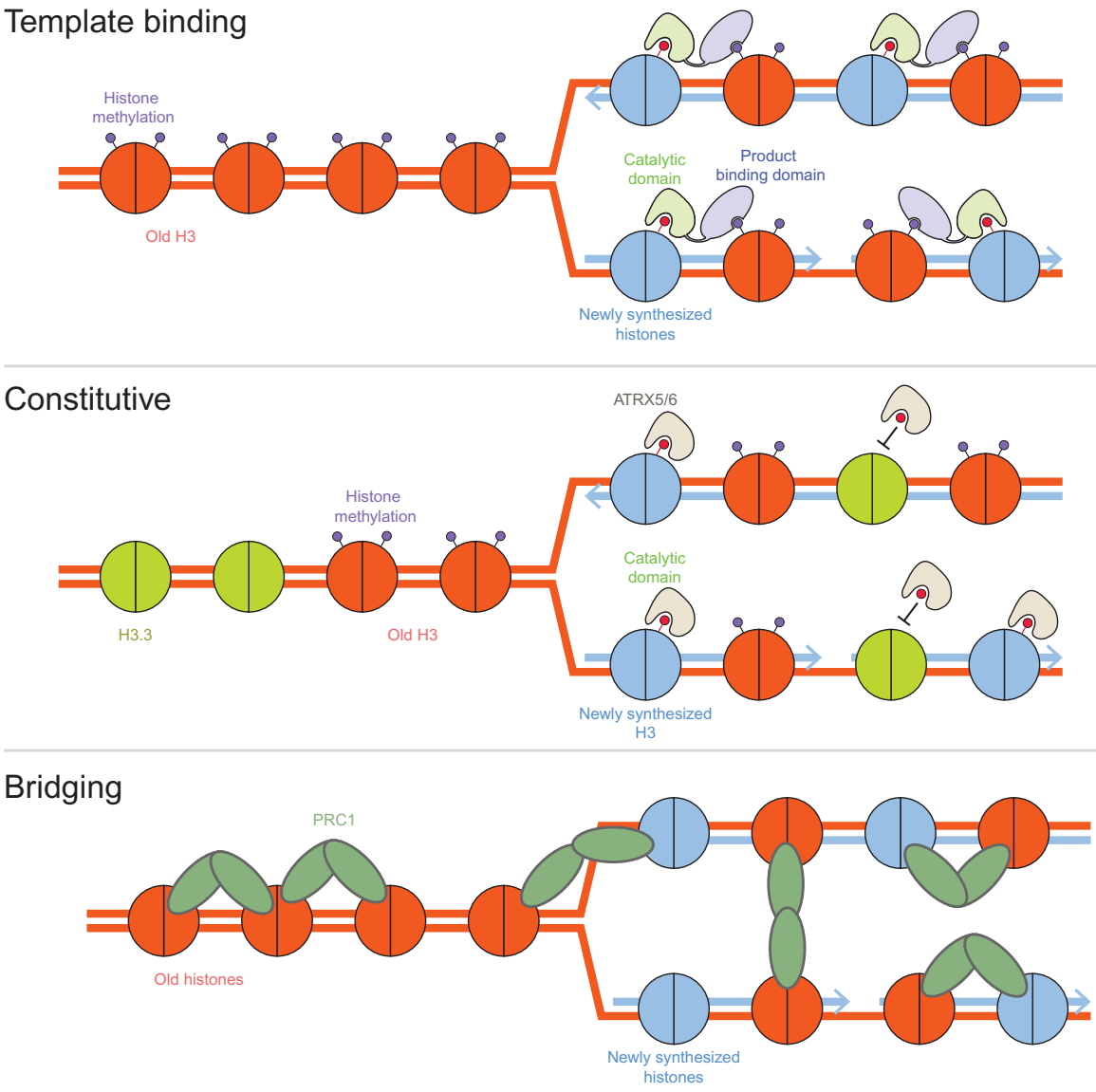

Fig. 2. Three models of propagating histone modifications through replication. In the template-binding model (*42*), adjacent nucleosomes are modified by a histone-modifying enzyme that binds the modified residue on a nearby tail. In the constitutive model (*47*), H3K27 methylation is restored by recognition of H3A31 but not H3.3T31 by ATXR5/6, such that only replication-coupled (H3) nucleosomes, not replication-independent (H3.3) nucleosomes, are methylated on H3K27. In the bridging model (*48*), PRC1 bridges nucleosomes across daughter chromatids.

nearby nucleosomes with newly synthesized histones, or alternately, Ccc1 could be required for protection of H3K27me3 deposited by PRC2 from demethylation. The deposition of H3K27me3 in newly synthesized histones has been shown to occur during the G_1-S transition in cultured fly cells (*44*) and at a slow rate over the whole cell cycle in human cultured cells (*23*). Thus, after dilution during replication, the old H3K27me3 in conjunction with the EED subunit of PRC2 might lead to the methylation of the same or nearby nucleosome tails containing unmethylated H3.

The levels and localization of PRC2 could play important roles in maintaining H3K27 methylation levels through replication as evidenced by the up-regulation of PRC2 subunits during S phase in human cells (*45*) and rapid localization of PRC2 components to newly replicated chromatin in fly embryos (*46*) and in human cells (*21*). Another possible way to maintain H3K27 methylation after replication at silent domains would be for the histone methyltransferase to travel with the replication fork and methylate newly assembled nucleosomes. This mechanism has been most definitively worked out in plants (*47*). Histone methyltransferases ATRX5/6 are essential for heterochromatin condensation in plants. The crystal structure of ATRX5 with H3 shows that one of the amino acid differences between H3 and H3.3, Ala^{31} (compared to Thr^{31} of H3.3, a difference that is conserved between plants and animals), is critical for recognition of the H3 tail by ATRX5/6. Replacement of alanine by threonine at position 31 of H3 abrogates the ability of ATRX5 to methylate H3. Thus, one of the four amino acid differences between H3 and H3.3 is used by ATRX5/6 to specifically methylate H3 and not H3.3. During replication, ATRX5 can monomethylate newly synthesized H3, whereas active regions

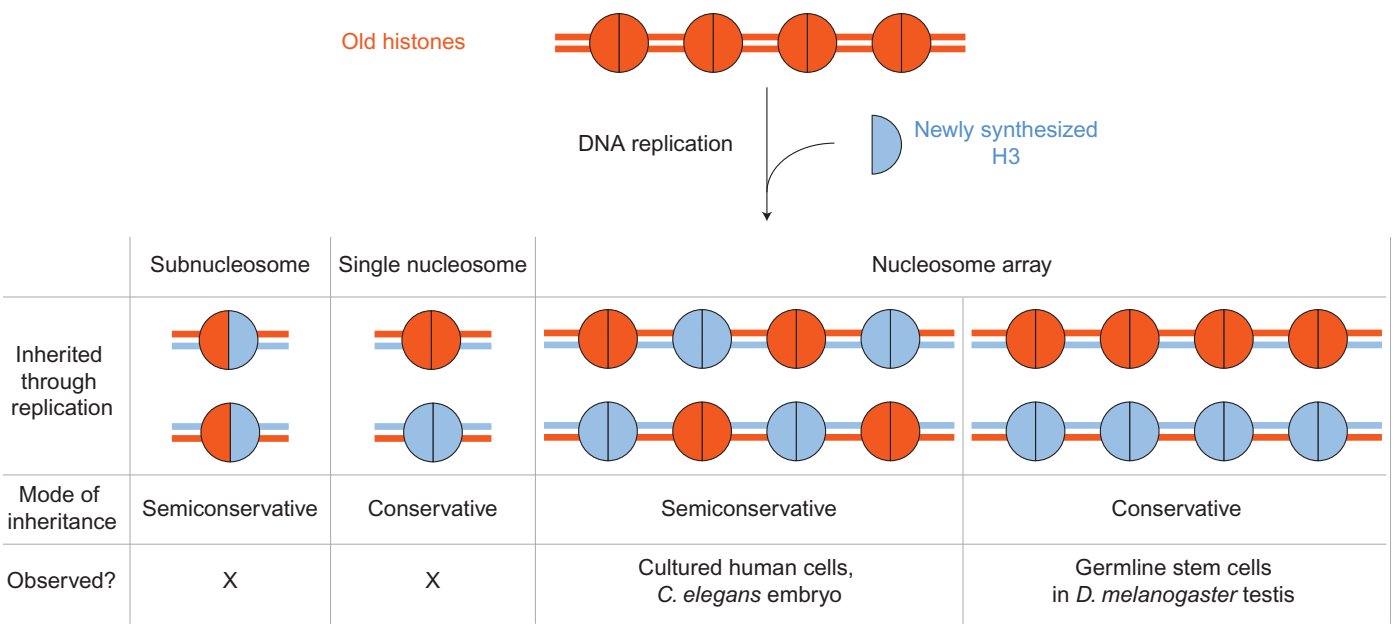

Fig. 3. Different modes of inheritance of old H3 and newly synthesized H3 on newly replicated DNA.

containing H3.3 are protected because of ATRX5/6's specificity for H3. Thus, a key histone modification that is needed for heterochromatin compaction is specified only by the replication-coupled H3 variant, where the methylated form of Lys^{27} is constitutively specified by Ala^{31} (Fig. 2). Notably, protein methyltransferases are still being discovered in metazoans, and a mechanism similar to that adopted by ATRX5/6 may yet be found in the animal kingdom.

Maintenance of chromatin states through replication could also be dependent on the action of non-histone chromatin proteins at the replication fork. The bridging model (*48*) requires a non-histone chromatin protein to have the ability both to bind nucleosomes and to oligomerize (Fig. 2). For example, PRC1 compacts chromatin by forming clusters with nucleosomes and other PRC1 complexes. In vitro, PRC1 clusters have been found to bridge old nucleosomes in front of the fork with newly assembled nucleosomes behind the fork, thus transferring the seed for compaction from the old chromosome to the daughter chromosome (*48*). PRC1 has been further shown to bridge the two daughter chromosomes also, which would ensure that the nucleosomes on the Okazaki fragment are also bound by PRC1 (Fig. 2), even though they are assembled by a fork that does not encounter PRC1 (*48*).

In summary, the dilution of posttranslationally modified histones during replication may be mitigated by distinct mechanisms in distinct contexts: (i) gradual replenishment during the remainder of the cell cycle, (ii) replenishment just during the G_1-S transition as seen in cultured animal cells, (iii) by concurrent modification of histones during replication fork movement as seen in plants, and (iv) bridging of non-histone chromatin proteins across the fork and between the two daughter chromosomes. The current mechanisms proposed for propagating nucleosome landscapes through replication can be encapsulated into three models: template binding, constitutive, and bridging (Fig. 2). In the template-binding model, the modifying machinery recognizes old nucleosomes and then modifies the nearby nucleosomes that contain newly synthesized histones. In the constitutive model that is exemplified by action of ATRX5/6 at the replication fork, the mode of assembly of H3 would determine its ultimate fate. This model posits that replication-independent assembly incorporates H3.3, which would acquire modifications associated with the chromatin state where it is being incorporated, whereas replication-coupled H3 is assembled by default in a neutral or silenced state. In the bridging model, non-histone chromatin proteins bridge replication forks and the two daughter chromosomes to maintain compact chromatin states through fork passage. The mechanisms of transfer of histones and non-histone proteins at replication forks will ultimately determine which model is involved in maintaining nucleosome landscapes at different genomic locations.

THE UNIT OF CHROMATIN THAT IS INHERITED THROUGH REPLICATION

The discontinuity and asymmetry of replication have implications for old nucleosome disruption and assembly of new nucleosomes onto daughter chromosomes. The helicase that unwinds DNA proceeds on the leading strand, on which DNA synthesis is performed by polymerase η (*49*). DNA unwinding leaves a lagging strand that is initially replicated by polymerase α and then by polymerase δ in discontinuous steps to produce the so-called Okazaki fragments (*49*). With respect to chromatin, leading strand synthesis involves nucleosome disruption, thus having first access to old histones, whereas Okazaki synthesis does not encounter old histones during fork passage. Thus, one could imagine an asymmetry in the extent of old histones in nucleosome arrays assembled by leading and lagging strands, with the old histones being retained on the leading strand and the new histones on the lagging strand. As multiple origins fire bidirectionally over the length of the chromosome, asymmetry between old nucleosome retention and new nucleosome assembly would average out over the whole chromosome. Early experiments suggested that under most circumstances, histones are symmetrically distributed between leading and lagging strands. Electron microscopy of S-phase chromatin from rapidly dividing

Drosophila blastoderm nuclei visualized closely spaced replication bubbles (*50*). In these images, one could not distinguish any difference in the presence of free DNA in the leading and lagging strand. All studies to date have detected no more than 200 bp of nucleosome-free DNA behind the replication fork. Furthermore, independent labeling of DNA and histones through several generations of cells in culture has shown that there is no preference for old histones on either leading or lagging strands (*51*). Thus, these studies suggest that there is no leading-versus-lagging strand bias in the distribution of old-versus-new nucleosomes.

DNA replication and CG methylation are both semiconservative processes, in which each parental strand templates its newly synthesized complement. In the case of nucleosomes, the issue of semiconservative versus conservative replication (Fig. 3) has been a matter of continuing debate since shortly after the discovery of nucleosomes in 1974 (*52*, *53*). By differentially labeling old and newly synthesized histones with dense amino acids, several studies have shown that octamers contain either only old histones or only new histones, that is, newly synthesized histones are not combined with old histones to form nucleosomes during replication-coupled assembly in bulk (*54*–*57*). The conservative transfer of histones was further narrowed down to H3-H4 specifically, when it was observed that old H2A-H2B could be part of nucleosomes that contained either old H3-H4 or newly synthesized H3-H4 (*58*, *59*). These studies were confirmed by an orthogonal method that involved incorporation of fluorescently labeled H3 in the species *Physarum microplasmodia* (*60*). When two labeled H3 molecules were part of the same nucleosome, there would be a fluorescence resonance energy transfer (FRET) signal. Initial incorporation resulted in nucleosomes containing two copies of labeled H3. Then, the fluorescence was tracked in the absence of any new incorporation of labeled H3, which would mean that after one cell cycle, all the labeled H3 would represent the old H3. Over several cell cycles, the FRET signal persisted over the fluorescence of a single H3, indicating that the old H3 remained in the same $(H3\text{-}H4)_2$ even after replication, confirming the bulk isotope labeling studies. Thus, H3-H4 is transferred conservatively during replication. In contrast to bulk chromatin, active chromatin has been characterized to have a mixture of old and new histones forming the octamer, indicating that nucleosome assembly during active processes independent of replication involves mixing of old and newly synthesized nucleosomes (*61*).

These early studies demonstrating a lack of mixing of old H3-H4 with newly synthesized H3-H4 were confirmed by differential labeling of old and new histones followed by mass spectrometry (MS), which offers an unambiguous identification of the proteins forming nucleosomes (*8*, *62*). Amino acid substitutions of H3 toward H3.3 are enough for it to be incorporated by replication-independent pathways, indicating the separation of incorporation pathways to be inherent to the histone variant. Compared to earlier studies in bulk that looked at transfer of old histones during replication, a major advance of the recent mass spectroscopy analysis was the ability to distinguish H3 and H3.3 (*62*). The MS analysis of newly assembled nucleosomes revealed that whenever a nucleosome contained an old H3 subunit, both H3 subunits were old. Thus, in line with the early studies, it was confirmed that the canonical H3 is not inherited through a half-nucleosome template. The same study found that 20% of the nucleosomes containing one old replication-independent variant H3.3 subunit contained a newly synthesized H3.3 as the other subunit. A follow-up study that mapped the genome-wide locations of nucleosomes containing one copy of old H3.3 and one copy of newly synthesized H3.3 found them predominantly at enhancers (*37*). The differential behavior of H3 and H3.3 is reflective of their distinct modes of incorporation into chromatin.

What can we conclude about replication-coupled nucleosome assembly based on the observation that individual nucleosomes generally replicate conservatively? One possibility is that old H3-H4 tetramers or whole octamers are transferred intact from the parental chromosome to the daughter chromosome (Fig. 3, single nucleosome). Another possibility is that nucleosomes are disassembled and reassembled to accommodate passage of the replication fork, which is suggested by the action of Asf1, a histone chaperone that binds an H3-H4 dimer and facilitates histone octamer disruption. The fact that the binding interface between Asf1 and H3-H4 precludes H3-H4 tetramer formation (*63*, *64*) and that Asf1 is required for fork passage (*65*) suggests that the histone octamer is disrupted ahead of the fork into histone dimers. Similar to Asf1, the other H3-H4 chaperone, Caf1, also binds H3-H4 dimers (*66*). Hence, although old nucleosomes are disrupted to dimers during fork passage, the old H3-H4 dimers do not mix with newly synthesized H3-H4 dimers when incorporated into nucleosomes. However, old and new histones make up equal parts of the newly replicated chromatin in bulk. Thus, nucleosomes with all-old H3-H4 or all-new H3-H4 could be assembled at random on each daughter chromatid, leading to equal distribution of nucleosomes with solely old H3-H4 or new H3-H4 over a nucleosome array (Fig. 3, semiconservative nucleosome array).

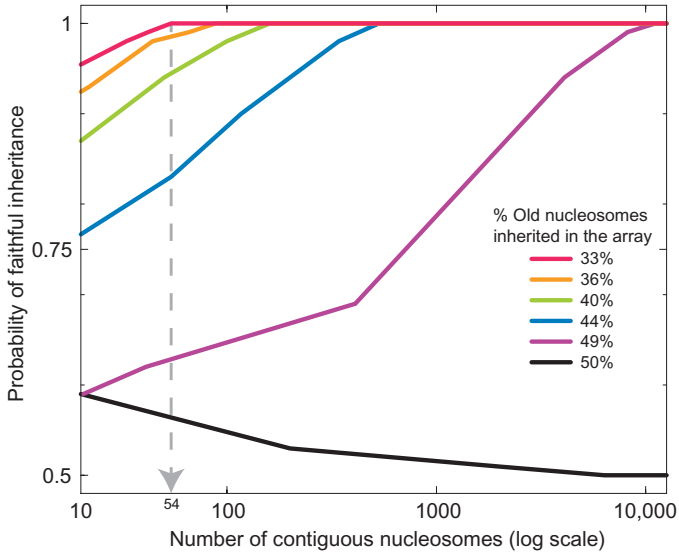

Fig. 4. Probability of faithful inheritance at various thresholds of old histone segregation. Given that old $(H3\text{-}H4)_2$ is segregated randomly to daughter chromosomes during replication, we can think of each nucleosome assembled as a Bernoulli trial, with the probability of a daughter chromosome assembling a nucleosome with old $(H3\text{-}H4)_2$ the same as the probability of assembling a nucleosome with new $(H3\text{-}H4)_2$, both of which would be 0.5. We can then ask, what is the probability that at least a given percentage of old $(H3\text{-}H4)_2$ in a nucleosome array of a given size is obtained by a daughter chromosome? The *x* axis of this plot represents the different nucleosome array sizes. The *y* axis represents the probability of the daughter chromosome getting at least a given percentage of old $(H3\text{-}H4)_2$. We define this binomial probability as the probability of faithful inheritance. The dashed gray line represents the minimum size of the nucleosome array that would ensure faithful inheritance of at least 33% of old $(H3\text{-}H4)_2$.

The inheritance of a mixture of old and new nucleosomes over a nucleosome array has implications for distinguishing between the template model and the assembly model. The minimal region required for stable inheritance of silenced domains through replication has been best characterized in flies, where PREs are required for stable silencing of homeotic genes through replication. PREs are ~700 bp, only enough for about three nucleosomes, but the silenced domains are in the scale of kilobases. If we look at the inheritance of nucleosomes at PREs alone through replication, based on the inheritance model we have described, during one of eight instances of the replication of a PRE, the newly replicated PRE will have all three nucleosomes consisting of newly synthesized H3-H4. Hence, if the epigenetic information were carried through old histones, this would be completely lost in one of eight times at the PRE (*67*). We can extend this idea to explore the minimum number of contiguous nucleosomes that could be inherited faithfully through replication. At maximum, only 50% of the time can daughter chromosomes faithfully acquire 50% or more of old nucleosomes (Fig. 4). Hence, 50% of the time, there will be loss of inheritance. At least 33% of old nucleosomes can be faithfully acquired by daughter chromosomes if the domain size is at least 54 nucleosomes (or ~10 kb). That is, 10 kb is the minimum size of a nucleosome domain in which at least 33% of nucleosomes can be faithfully inherited. This estimate agrees with the size of silent domains that are maintained around PREs through several rounds of replication during fly development. So for replication-coupled H3, the template model could operate over regions that span tens of kilobases of DNA and would involve the faithful inheritance of ~40% or fewer of old nucleosomes every time that region is replicated. In contrast, the template model could apply at a single nucleosome level for H3.3, where old H3.3 mixes with new H3.3 to form single nucleosomes at least 20% of the time in bulk. The possibility of such inheritance is highlighted by the fact that the mixing of old and new H3.3 occurs at cell type–specific enhancers (*37*) and that H3.3 is enriched at PREs relative to surrounding regions (*68*).

The conservative inheritance of H3, together with the high probability that old histones with modifications would be lost at short segments, argues against the simplest version of the template-binding model for short regulatory sequences. In contrast, the constitutive model is not affected by any of the constraints of the replication-coupled histone transfer process, because all H3s, but no H3.3s, will be modified. The bridging model remains an attractive possibility that is not mutually exclusive with the constitutive model, insofar as recruitment of PRC1 is known not to depend on H3K27 methylation (*69*, *70*).

CONCLUSION

We have described the diverse cellular strategies that are observed in the reconstitution of the steady-state nucleosome landscape after disruption and dilution of old nucleosomes during replication. In organisms with large and complex genomes, where a particular cell type requires that only a fraction of the genome is active, there needs to be a means of packaging and repressing the inactive majority of the genome during every cell division. To accomplish this feat during the brief passage of the replication fork, a choreographed process has evolved around replication-coupled assembly to propagate the silent state. This involves clusters of replication-coupled histone genes that are highly transcribed only during S phase, stem-loop binding protein, which protects the 3′ ends of histone mRNAs from cleavage and polyadenylation to channel histone mRNA into the specialized U7 SNURP RNA 3′-end processing machinery, and a dedicated replication-coupled nucleosome assembly pathway (*71*, *72*). Outside of replication, nucleosome assembly relies on the replication-independent H3.3 variant, which is incorporated when nucleosomes are lost due to either active processes or the inability of the underlying DNA to stably wrap nucleosomes. Yeast lacks H3K27 methylation, and the H3 variant is absent, so that a single H3.3-like variant has become the substrate for both replication-coupled and replication-independent pathways.

Apart from an inherent tendency of replication-coupled assembly to impose basal silencing, inheritance of posttranslationally modified histones at silent domains could play additional roles in maintenance of silent states. However, as we have discussed above, inheritance of old H3 alone is sufficiently reliable only for large chromatin domains (tens of kilobases) due to the histones being randomly transferred to daughter chromosomes during replication. Alternatively, by splitting H3.3-containing nucleosomes, inheritance of old H3.3 could be sufficient to maintain chromatin states at short regulatory sequences through replication. We anticipate that future research will focus on the extent of inheritance of old H3 required for stable maintenance of epigenetic states and the molecular mechanisms involved in inheritance of histone modifications when nucleosomes with one old H3.3 and one new H3.3 are formed.

REFERENCES AND NOTES

1. T. K. Kim, M. Hemberg, J. M. Gray, A. M. Costa, D. M. Bear, J. Wu, D. A. Harmin, M. Laptewicz, K. Barbara-Haley, S. Kuersten, E. Markenscoff-Papadimitriou, D. Kuhl, H. Bito, P. F. Worley, G. Kreiman, M. E. Greenberg, Widespread transcription at neuronal activity-regulated enhancers. *Nature* **465**, 182–187 (2010).
2. A. Mohd-Sarip, F. Venturini, G. E. Chalkley, C. P. Verrijzer, Pleiohomeotic can link polycomb to DNA and mediate transcriptional repression. *Mol. Cell. Biol.* **22**, 7473–7483 (2002).
3. R. B. Deal, J. G. Henikoff, S. Henikoff, Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. *Science* **328**, 1161–1164 (2010).
4. Y. V. Fu, H. Yardimci, D. T. Long, T. V. Ho, A. Guainazzi, V. P. Bermudez, J. Hurwitz, A. van Oijen, O. D. Schärer, J. C. Walter, Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. *Cell* **146**, 931–941 (2011).
5. V. Böhm, A. R. Hieb, A. J. Andrews, A. Gansen, A. Rocker, K. Tóth, K. Luger, J. Langowski, Nucleosome accessibility governed by the dimer/tetramer interface. *Nucleic Acids Res.* **39**, 3093–3102 (2011).
6. L. Louters, R. Chalkley, Exchange of histones H1, H2A, and H2B in vivo. *Biochemistry* **24**, 3080–3085 (1985).
7. E. McKittrick, P. R. Gafken, K. Ahmad, S. Henikoff, Histone H3.3 is enriched in covalent modifications associated with active chromatin. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1525–1530 (2004).
8. K. Ahmad, S. Henikoff, The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191–1200 (2002).
9. S. Enomoto, P. D. McCune-Zierath, M. Gerami-Nejad, M. A. Sanders, J. Berman, *RLF2*, a subunit of yeast chromatin assembly factor-I, is required for telomeric chromatin function in vivo. *Genes Dev.* **11**, 358–370 (1997).
10. P. D. Kaufman, R. Kobayashi, B. Stillman, Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I. *Genes Dev.* **11**, 345–357 (1997).
11. E. K. Monson, D. de Bruin, V. A. Zakian, The yeast Cac1 protein is required for the stable inheritance of transcriptionally repressed chromatin at telomeres. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13081–13086 (1997).
12. S. Enomoto, J. Berman, Chromatin assembly factor I contributes to the maintenance, but not the re-establishment, of silencing at the yeast silent mating loci. *Genes Dev.* **12**, 219–232 (1998).
13. S. Nakano, B. Stillman, H. R. Horvitz, Replication-coupled chromatin assembly generates a neuronal bilateral asymmetry in *C. elegans*. *Cell* **147**, 1525–1536 (2011).
14. H. Kaya, K. I. Shibahara, K. I. Taoka, M. Iwabuchi, B. Stillman, T. Araki, *FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. *Cell* **104**, 131–142 (2001).

15. T. Ono, H. Kaya, S. Takeda, M. Abe, Y. Ogawa, M. Kato, T. Kakutani, S. O. Mittelsten, T. Araki, K. Shibahara, Chromatin assembly factor 1 ensures the stable maintenance of silent chromatin states in *Arabidopsis*. *Genes Cells* **11**, 153–162 (2006).
16. Y. Song, F. He, G. Xie, X. Guo, Y. Xu, Y. Chen, X. Liang, I. Stagljar, D. Egli, J. Ma, R. Jiao, CAF-1 is essential for *Drosophila* development and involved in the maintenance of epigenetic memory. *Dev. Biol.* **311**, 213–222 (2007).
17. B. Klapholz, B. H. Dietrich, C. Schaffner, F. Hérédia, J. P. Quivy, G. Almouzni, N. Dostatni, CAF-1 is required for efficient replication of euchromatic DNA in *Drosophila* larval endocycling cells. *Chromosoma* **118**, 235–248 (2009).
18. D. Ray-Gallet, A. Woolfe, I. Vassias, C. Pellentz, N. Lacoste, A. Puri, D. C. Schultz, N. A. Pchelintsev, P. D. Adams, L. E. T. Jansen, G. Almouzni, Dynamics of histone H3 deposition in vivo reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity. *Mol. Cell* **44**, 928–941 (2011).
19. M. Bui, M. P. Walkiewicz, E. K. Dimitriadis, Y. Dalal, The CENP-A nucleosome: A battle between Dr Jekyll and Mr Hyde. *Nucleus* **4**, 37–42 (2013).
20. C. Alabert, J. C. Bukowski-Wills, S. B. Lee, G. Kustatscher, K. Nakamura, A. F. de Lima, P. Menard, J. Mejlvang, J. Rappsilber, A. Groth, Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat. Cell Biol.* **16**, 281–293 (2014).
21. K. H. Leung, M. Abou El Hassan, R. Bremner, A rapid and efficient method to purify proteins at replication forks under native conditions. *Biotechniques* **55**, 204–206 (2013).
22. B. M. Sirbu, F. B. Couch, J. T. Feigerle, S. Bhaskara, S. W. Hiebert, D. Cortez, Analysis of protein dynamics at active, stalled, and collapsed replication forks. *Genes Dev.* **25**, 1320–1327 (2011).
23. C. Alabert, T. K. Barth, N. Reverón-Gómez, S. Sidoli, A. Schmidt, O. N. Jensen, A. Imhof, A. Groth, Two distinct modes for propagation of histone PTMs across the cell cycle. *Genes Dev.* **29**, 585–590 (2015).
24. J. Müller, C. M. Hart, N. J. Francis, M. L. Vargas, A. Sengupta, B. Wild, E. L. Miller, M. B. O'Connor, R. E. Kingston, J. A. Simon, Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell* **111**, 197–208 (2002).
25. A. Kuzmichev, K. Nishioka, H. Erdjument-Bromage, P. Tempst, D. Reinberg, Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev.* **16**, 2893–2905 (2002).
26. L. Ringrose, R. Paro, Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu. Rev. Genet.* **38**, 413–443 (2004).
27. L. J. Gaydos, W. Wang, S. Strome, Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science* **345**, 1515–1518 (2014).
28. V. Tran, C. Lim, J. Xie, X. Chen, Asymmetric division of *Drosophila* male germline stem cell shows asymmetric histone distribution. *Science* **338**, 679–682 (2012).
29. P. Hemmerich, S. Weidtkamp-Peters, C. Hoischen, L. Schmiedeberg, I. Erliandri, S. Diekmann, Dynamics of inner kinetochore assembly and maintenance in living cells. *J. Cell Biol.* **180**, 1101–1114 (2008).
30. J. Wisniewski, B. Hajj, J. Chen, G. Mizuguchi, H. Xiao, D. Wei, M. Dahan, C. Wu, Imaging the fate of histone Cse4 reveals de novo replacement in S phase and subsequent stable residence at centromeres. *Elife* **3**, e02203 (2014).
31. P. Drané, K. Ouararhni, A. Depaux, M. Shuaib, A. Hamiche, The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. *Genes Dev.* **24**, 1253–1265 (2010).
32. P. W. Lewis, S. J. Elsaesser, K. M. Noh, S. C. Stadler, C. D. Allis, Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14075–14080 (2010).
33. D. Ray-Gallet, J. P. Quivy, C. Scamps, E. M. Martini, M. Lipinski, G. Almouzni, HIRA is critical for a nucleosome assembly pathway independent of DNA synthesis. *Mol. Cell* **9**, 1091–1100 (2002).
34. Y. Mito, J. G. Henikoff, S. Henikoff, Genome-scale profiling of histone H3.3 replacement patterns. *Nat. Genet.* **37**, 1090–1097 (2005).
35. C. M. Weber, J. G. Henikoff, S. Henikoff, H2A.Z nucleosomes enriched over active genes are homotypic. *Nat. Struct. Mol. Biol.* **17**, 1500–1507 (2010).
36. G. Hu, D. E. Schones, K. Cui, R. Ybarra, D. Northrup, Q. Tang, L. Gattinoni, N. P. Restifo, S. Huang, K. Zhao, Regulation of nucleosome landscape and transcription factor targeting at tissue-specific enhancers by BRG1. *Genome Res.* **21**, 1650–1658 (2011).
37. C. Huang, Z. Zhang, M. Xu, Y. Li, Z. Li, Y. Ma, T. Cai, B. Zhu, H3.3-H4 tetramer splitting events feature cell-type specific enhancers. *PLOS Genet.* **9**, e1003558 (2013).
38. J. I. Schneiderman, A. Sakai, S. Goldstein, K. Ahmad, The XNP remodeler targets dynamic chromatin in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14472–14477 (2009).
39. J. I. Schneiderman, G. A. Orsi, K. T. Hughes, B. Loppin, K. Ahmad, Nucleosome-depleted chromatin gaps recruit assembly factors for the H3.3 histone variant. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 19721–19726 (2012).
40. A. D. Goldberg, L. A. Banaszynski, K.-M. Noh, P. W. Lewis, S. J. Elsaesser, S. Stadler, S. Dewell, M. Law, X. Guo, X. Li, D. Wen, A. Chapgier, R. C. DeKelver, J. C. Miller, Y.-L. Lee, E. A. Boydston, M. C. Holmes, P. D. Gregory, J. M. Greally, S. Rafii, C. Yang, P. J. Scambler, D. Garrick, R. J. Gibbons, D. R. Higgs, I. M. Cristea, F. D. Urnov, D. Zheng, C. D. Allis, Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* **140**, 678–691 (2010).
41. H. P. Voon, J. R. Hughes, C. Rode, I. A. De LaRosa-Velázquez, T. Jenuwein, R. Feil, D. R. Higgs, R. J. Gibbons, ATRX plays a key role in maintaining silencing at interstitial heterochromatic loci and imprinted genes. *Cell Rep.* **11**, 405–418 (2015).
42. R. Margueron, N. Justin, K. Ohno, M. L. Sharpe, J. Son, W. J. Drury III, P. Voigt, S. R. Martin, W. R. Taylor, V. De Marco, V. Pirrotta, D. Reinberg, S. J. Gamblin, Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* **461**, 762–767 (2009).
43. P. A. Dumesic, C. M. Homer, J. J. Moresco, L. R. Pack, E. K. Shanle, S. M. Coyle, B. D. Strahl, D. G. Fujimori, J. R. Yates III, H. D. Madhani, Product binding enforces the genomic specificity of a yeast polycomb repressive complex. *Cell* **160**, 204–218 (2015).
44. C. Lanzuolo, F. Lo Sardo, A. Diamantini, V. Orlando, PcG complexes set the stage for epigenetic inheritance of gene silencing in early S phase before replication. *PLOS Genet.* **7**, e1002370 (2011).
45. A. P. Bracken, D. Pasini, M. Capra, E. Prosperini, E. Colli, K. Helin, *EZH2* is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* **22**, 5323–5335 (2003).
46. S. Petruk, Y. Sedkov, D. M. Johnston, J. W. Hodgson, K. L. Black, S. K. Kovermann, S. Beck, E. Canaani, H. W. Brock, A. Mazo, TrxG and PcG proteins but not methylated histones remain associated with DNA through replication. *Cell* **150**, 922–933 (2012).
47. Y. Jacob, E. Bergamin, M. T. A. Donoghue, V. Mongeon, C. LeBlanc, P. Voigt, C. J. Underwood, J. S. Brunzelle, S. D. Michaels, D. Reinberg, J.-F. Couture, R. A. Martienssen, Selective methylation of histone H3 variant H3.1 regulates heterochromatin replication. *Science* **343**, 1249–1253 (2014).
48. S. M. Lo, N. E. Follmer, B. M. Lengsfeld, E. V. Madamba, S. Seong, D. J. Grau, N. J. Francis, A bridging model for persistence of a polycomb group protein complex through DNA replication in vitro. *Mol. Cell* **46**, 784–796 (2012).
49. M. A. Reijns, H. Kemp, J. Ding, S. M. de Procé, A. P. Jackson, M. S. Taylor, Lagging-strand replication shapes the mutational landscape of the genome. *Nature* **518**, 502–506 (2015).
50. S. L. McKnight, O. L. Miller Jr., Electron microscopic analysis of chromatin replication in the cellular blastoderm *Drosophila melanogaster* embryo. *Cell* **12**, 795–804 (1977).
51. E. Fowler, R. Farb, S. El-Saidy, Distribution of the core histones H2A.H2B.H3 and H4 during cell replication. *Nucleic Acids Res.* **10**, 735–748 (1982).
52. R. D. Kornberg, J. O. Thomas, Chromatin structure: Oligomers of the histones. *Science* **184**, 865–868 (1974).
53. A. L. Olins, D. E. Olins, Spheroid chromatin units (v bodies). *Science* **183**, 330–332 (1974).
54. I. M. Leffak, Stability of the conservative mode of nucleosome assembly. *Nucleic Acids Res.* **11**, 2717–2732 (1983).
55. I. M. Leffak, Conservative segregation of nucleosome core histones. *Nature* **307**, 82–85 (1984).
56. I. M. Leffak, R. Grainger, H. Weintraub, Conservative assembly and segregation of nucleosomal histones. *Cell* **12**, 837–845 (1977).
57. H. Weintraub, S. J. Flint, I. M. Leffak, M. Groudine, R. M. Grainger, The generation and propagation of variegated chromosome structures. *Cold Spring Harb. Symp. Quant. Biol.* **42** (Pt. 1), 401 (1978).
58. K. Yamasu, T. Senshu, Conservative segregation of tetrameric units of H3 and H4 histones during nucleosome replication. *J. Biochem.* **107**, 15–20 (1990).
59. V. Jackson, Deposition of newly synthesized histones: New histones H2A and H2B do not deposit in the same nucleosome with new histones H3 and H4. *Biochemistry* **26**, 2315–2325 (1987).
60. C. P. Prior, C. R. Cantor, E. M. Johnson, V. G. Allfrey, Incorporation of exogenous pyrene-labeled histone into Physarum chromatin: A system for studying changes in nucleosomes assembled in vivo. *Cell* **20**, 597–608 (1980).
61. S. Kumar, M. Leffak, Assembly of active chromatin. *Biochemistry* **25**, 2055–2060 (1986).
62. M. Xu, C. Long, X. Chen, C. Huang, S. Chen, B. Zhu, Partitioning of histone H3-H4 tetramers during DNA replication–dependent chromatin assembly. *Science* **328**, 94–98 (2010).
63. C. M. English, M. W. Adkins, J. J. Carson, M. E. Churchill, J. K. Tyler, Structural basis for the histone chaperone activity of Asf1. *Cell* **127**, 495–508 (2006).
64. R. Natsume, M. Eitoku, Y. Akai, N. Sano, M. Horikoshi, T. Senda, Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4. *Nature* **446**, 338–341 (2007).
65. A. Groth, A. Corpet, A. J. Cook, D. Roche, J. Bartek, J. Lukas, G. Almouzni, Regulation of replication fork progression through histone supply and demand. *Science* **318**, 1928–1931 (2007).
66. H. Tagami, D. Ray-Gallet, G. Almouzni, Y. Nakatani, Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* **116**, 51–61 (2004).
67. S. Henikoff, T. Furuyama, K. Ahmad, Histone variants, nucleosome assembly and epigenetic inheritance. *Trends Genet.* **20**, 320–326 (2004).

68. Y. Mito, J. G. Henikoff, S. Henikoff, Histone replacement marks the boundaries of cis-regulatory domains. *Science* **315**, 1408–1411 (2007).
69. S. Cooper, M. Dienstbier, R. Hassan, L. Schermelleh, J. Sharif, N. P. Blackledge, V. De Marco, S. Elderkin, H. Koseki, R. Klose, A. Heger, N. Brockdorff, Targeting polycomb to pericentric heterochromatin in embryonic stem cells reveals a role for H2AK119u1 in PRC2 recruitment. *Cell Rep.* **7**, 1456–1470 (2014).
70. N. P. Blackledge, A. M. Farcas, T. Kondo, H. W. King, J. F. McGouran, L. L. Hanssen, S. Ito, S. Cooper, K. Kondo, Y. Koseki, T. Ishikura, H. K. Long, T. W. Sheahan, N. Brockdorff, B. M. Kessler, H. Koseki, R. J. Klose, Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. *Cell* **157**, 1445–1459 (2014).
71. Z. Dominski, L. X. Zheng, R. Sanchez, W. F. Marzluff, Stem-loop binding protein facilitates 3′-end formation by stabilizing U7 snRNP binding to histone pre-mRNA. *Mol. Cell. Biol.* **19**, 3561–3570 (1999).
72. W. F. Marzluff, E. J. Wagner, R. J. Duronio, Metabolism and regulation of canonical histone mRNAs: Life without a poly(A) tail. *Nat. Rev. Genet.* **9**, 843–854 (2008).

Acknowledgments: We thank P. Talbert for the critical reading of the manuscript. **Funding:** Support is from the Howard Hughes Medical Institute. **Author contributions:** S.R. and S.H. wrote the paper. **Competing interests:** The authors declare that they have no conflicts of interest.

Submitted 10 May 2015
Accepted 24 June 2015
Published 7 August 2015
10.1126/sciadv.1500587

Citation: S. Ramachandran, S. Henikoff, Replicating nucleosomes. *Sci. Adv.* **1**, e1500587 (2015).

Replicating nucleosomes

Srinivas Ramachandran and Steven Henikoff

Sci Adv **1** (7), e1500587.
DOI: 10.1126/sciadv.1500587