Trends in Genetics

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Review

At the fork: where epigenetic decisions are made

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Epigenetic inheritance ensures the transmission of chromatin states through cell division, despite the dilution of parental histones during DNA replication. Recent studies reveal that inheritance is not a passive process but instead involves regulated, replication-coupled decisions coordinated by the replisome, histone chaperones, and histone modification feedback loops. In this review, I synthesize recent advances in histone recycling and chromatin inheritance, highlighting how regulated parental histone segregation underpins robust epigenetic memory. I propose a conceptual framework that positions the replication fork as an epigenetic decision-making hub and highlight key unresolved questions that will shape future studies.

Highlights

Parental histone segregation is an active, replisome-coupled process that preserves chromatin states during DNA replication.

Histone disassembly, capture, retention, and redeposition are coordinated with newly synthesized histones to balance continuity and plasticity.

Strand-specific recycling, positional memory, and histone variant handling modulate epigenetic inheritance.

Feedback loops between histone modifications and modifying enzymes stabilize partial inheritance into robust chromatin states.

Chromatin-based epigenetic inheritance in the context of DNA replication

Eukaryotic DNA is folded with histone proteins into chromatin, and the covalent post-translational modifications (PTMs) of histones play critical roles in regulating every aspect of DNA metabolism, such as transcription, replication, and DNA damage repair [1]. The faithful transmission of chromatin states is essential for genome regulation, development, and the maintenance of cell identity [2]. Historically, models of chromatin-based epigenetic inheritance have centered on the 'read–write' cycle on histones: during DNA replication, parental histones are distributed to daughter strands at their original locations and mixed with newly synthesized histones; then, through a self-reinforcing mechanism, histone-modifying enzymes recognize pre-existing PTMs and catalyze the same PTMs on newly incorporated histones [3]. In this paradigm, parental histones serve as essential 'seeds' for the restoration of chromatin domains following replication.

However, the physical process of DNA replication poses a profound challenge to chromatin stability. Nucleosomes are disrupted ahead of the replication fork, parental histones are redistributed between daughter strands, and newly synthesized, largely unmodified histones are incorporated [2,4]. This inherently disruptive process raises a critical question: how is sufficient parental information retained at the correct genomic locations to ensure faithful chromatin inheritance without being lost through stochastic diffusion?

Recent work has reframed epigenetic inheritance as an active, replication-coupled process rather than a passive consequence of DNA replication [2,4]. This conceptual shift has been driven by three major developments in the last decade. First, strand-specific approaches such as eSPAN and SCAR-seq have enabled direct analysis of parental histone segregation to leading and lagging strands [5–8]. Second, the identification of replisome-associated histone-binding proteins [5,9–16] has provided powerful points of experimental manipulation, yielding mechanistic insight into histone recycling at replication forks. Third, advances in eukaryotic replisome reconstitution [17–19] have enabled cryo-electron microscopy (cryo-EM) studies that reveal the spatial organization of replication machinery at near-atomic resolution [20–26].

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Together, these studies have established the replisome as a highly coordinated hub that integrates helicases, polymerases, and histone chaperones into a single, dynamic architecture [4,27,28]. Rather than being a simple copying mechanism, epigenetic inheritance is now better viewed as a series of fork-associated decisions: when and how parental nucleosomes are dismantled, which histones are retained, where they are redeposited, and how their modifications are interpreted following replication.

Rather than exhaustively summarizing the field, this review focuses on the unresolved questions that define these processes. Although many molecular players have been identified, the principles governing their coordination and regulation remain unclear. I use this framework to highlight key conceptual gaps and outline the major challenges in understanding chromatin-based inheritance.

Current understanding of parental histone segregation and the read–write cycle

Parental histone segregation

A central question in epigenetic inheritance is deceptively simple: what information survives DNA replication? Biochemical and metabolic labeling studies have established that, whereas H2A–H2B dimers exchange readily, H3–H4 tetramers largely remain intact during DNA replication [29]. These findings position the H3–H4 tetramer as the primary carrier of epigenetic information, with PTMs on parental H3–H4 providing instructive cues for post-replicative chromatin restoration.

Strand-specific approaches demonstrated that parental histones are distributed in a symmetric manner on leading and lagging strands. Importantly, this symmetry emerges from active regulation rather than stochastic redistribution, as mutations in distinct replisome-associated proteins can bias the segregation into a single strand [2,4].

On the leading strand, histone recycling is dominated by DNA polymerase ε. The Dpb3/Dpb4 subunits (POLE3/POLE4 in mammals) bind H3–H4 and promote their efficient redeposition [5,30,31] (Figure 1). Whether additional proteins contribute to this process remains unclear. Structural analyses indicate that polymerase ε can adopt flexible conformations compatible with both capturing histones displaced by the advancing helicase and redepositing them onto newly synthesized DNA [23,25,32]. The continuous nature of leading-strand synthesis likely facilitates tight coupling between histone eviction and redeposition.

By contrast, parental H3–H4 transfer to the lagging strand involves a broader and more distributed network of histone-binding activities (Figure 1). These include the Mcm2 subunit of the MCM helicase, the largest subunit of DNA polymerase α, the Pol32 subunit of DNA polymerase δ, and Mrc1/CLASPIN of the fork protection complex (FPC). In addition, proper transfer also requires structural replisome components such as Ctf4/AND1 and PCNA [5–7,30,31,33–39]. The engagement of multiple factors likely reflects the discontinuous nature of lagging-strand synthesis and the temporal separation between histone eviction and redeposition. They also support a relay model in which parental H3–H4 are sequentially handed off from histone-binding proteins positioned near the site of nucleosome disassembly, such as Mcm2, to factors associated with sites of DNA synthesis, including polymerases α and δ [34].

Together, these studies reveal that parental histone segregation is tightly integrated with the architecture and dynamics of the replication fork. Understanding epigenetic inheritance now requires moving beyond cataloging individual factors to examining how the replisome integrates competing interactions and timing constraints to determine inheritance outcomes.

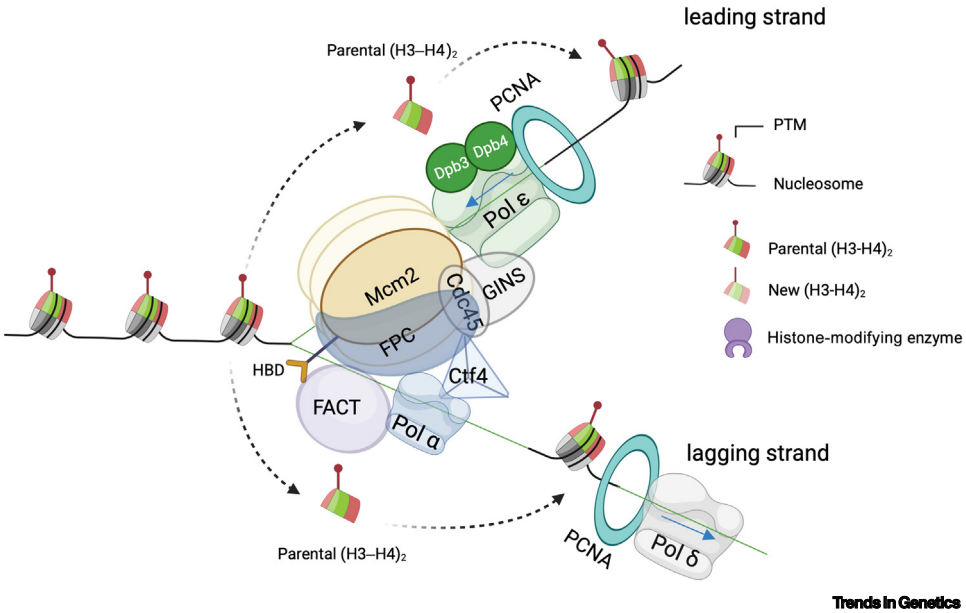

Figure 1. Schematic diagram parental histone segregation and read–write cycle. Created in BioRender. https://BioRender.com/80jwdcl. FACT: facilitates chromatin transcription; FPC: fork protection complex; GINS: Go-Ichi-Ni-San; HBD: histone binding domain; PCNA: proliferating cell nuclear antigen; PTM: post-translational modification.

The read–write cycle

Parental H3–H4 histones occupy only approximately half of the nucleosomal positions on replicated DNA, with newly synthesized, largely unmodified histones filling the remainder [2,40]. DNA replication, therefore, imposes an intrinsic twofold dilution of histone modification density. As a result, parental histone segregation alone cannot preserve chromatin states at full strength and must be complemented by enzymatic reinforcement through PTM-dependent recruitment mechanisms [2].

The classical framework for such reinforcement is the 'read–write' cycle, in which histone-modifying enzymes both recognize and propagate specific histone marks [3]. In this model, pre-existing modifications on parental histones are 'read' by dedicated binding domains, which recruit or activate the same enzymes that 'write' these modifications onto adjacent, newly incorporated histones. Through iterative cycles of recognition and catalysis, local chromatin environments can be restored following the disruptive passage of the replication fork.

Canonical examples of such reinforcement are found in heterochromatin. The H3K9 methyltransferases Clr4/SUV39 couple catalytic activity with chromodomain-mediated recognition of H3K9me3 [41,42], while the H3K27 methyltransferase PRC2 relies on the EED subunit to bind H3K27me3 [43]. This principle has now been extended to euchromatin, where the H3K4 methyltransferase COMPASS uses the Spp1 subunit to recognize H3K4me3 [44]. In each case, parental histone modifications provide a recruitment platform for histone-modifying enzymes to restore chromatin states following replication.

Together, these examples establish the read–write cycle as a central mechanism for reinforcing different types of chromatin states following replication, complementing parental histone segregation to restore epigenetic information on newly synthesized DNA.

Functional studies

Functional studies provide an important link between the molecular mechanisms of parental histone segregation and their biological consequences. Disruption of histone recycling pathways causes relatively modest defects in heterochromatin in budding yeast, a classical model of chromatin-based inheritance [5,45]. However, careful analysis of fission yeast heterochromatin suggests that these mild phenotypes reflect the ability of heterochromatin to be established *de novo* [30]. When *de novo* establishment pathways are compromised, defects in parental histone segregation, particularly along the lagging strand, lead to pronounced failures in chromatin inheritance [30].

In mammalian systems, compromising parental histone segregation pathways has been shown to disproportionately affect stem cell differentiation and result in embryonic lethality [46–49]. These phenotypes likely reflect the heightened sensitivity of developmental gene regulatory programs to perturbations in replication-coupled chromatin inheritance. In this context, even subtle defects in histone recycling may lead to cumulative errors in chromatin-state propagation, ultimately altering transcriptional programs and cell fate decisions.

Together, these studies highlight that the functional importance of parental histone segregation is highly context dependent, becoming particularly evident in systems where chromatin states must be stably maintained over multiple cell divisions. At the same time, they underscore that our understanding of the physiological consequences of replication-coupled chromatin inheritance remains limited. As experimental approaches continue to improve, particularly in multicellular and developmental systems, further studies will be essential to define how defects in histone segregation contribute to gene regulation, cell identity, and disease.

Decision-making at the replication fork: unresolved principles of epigenetic inheritance

Recent findings suggest that epigenetic inheritance emerges from coordinated decision-making at the replication fork, where multiple competing processes are integrated in space and time. The replisome is uniquely positioned to function as such a regulatory hub, assembling DNA polymerases, helicases, scaffold proteins, histone chaperones, and chromatin-associated factors into a highly organized yet dynamic structure [27,28]. This view naturally raises a set of unresolved questions concerning how decisions are made, enforced, and modulated during DNA replication (see Outstanding questions; Figure 2).

What is the unit of inheritance?

A fundamental question in replication-coupled inheritance concerns the physical form in which parental chromatin information is transmitted. Early biochemical studies established the H3–H4 tetramer as a stable unit during DNA replication [29], positioning it as the primary carrier of epigenetic information. However, recent structural work suggests a more complex picture. The histone chaperone facilitates chromatin transcription (FACT) and the Mcm2 subunit of the helicase have been observed to associate with a histone hexamer, composed of one H3–H4 tetramer and one H2A–H2B dimer [32,50]. Similar hexamer intermediates have been observed in structural studies of FACT with transcription elongation complexes, supporting their biological relevance beyond replication [51]. In parallel, evidence that H2A–H2B modifications can be inherited during DNA replication is consistent with the possibility of hexamer-based transmission [52,53].

Whether these hexamers represent transient intermediates or functionally meaningful units of inheritance remains unclear, and both models remain compatible with the observation that H3–H4 tetramers do not split during replication. Defining the physical unit of inheritance will be

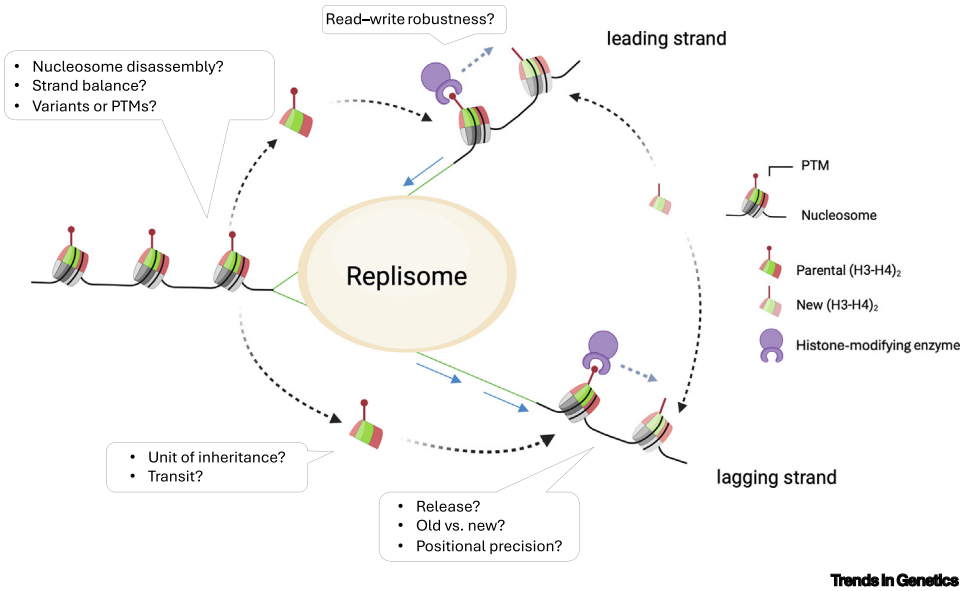

Figure 2. Schematic diagram of the replisome as a decision hub. Created in BioRender. https://BioRender.com/gbv49db. PTM: post-translational modification.

essential for understanding what information is preserved and how it is propagated through DNA replication.

How are nucleosomes disassembled ahead of the fork?

The first decision point in parental histone segregation is how nucleosomes are dismantled as they encounter the advancing replisome. Histone chaperones are central to this process [54]. FACT associates with both the replisome and partially unwrapped nucleosomal intermediates and can stabilize histone-DNA interactions while permitting DNA translocation [51,55–58]. Its enrichment at replication forks suggests that nucleosome disassembly is actively coordinated to preserve parental histones in recycling-competent forms and prevent their dissociation into the nucleoplasmic pool [11,32,59,60].

An unresolved question is how chromatin context influences nucleosome disassembly. Heterochromatin, characterized by high nucleosome density and the presence of chromatin-binding factors, may resist complete unwrapping or impose alternative disassembly kinetics. Histone variants and PTMs could further modulate nucleosome stability, biasing the fate of parental histones prior to their full release from DNA. Thus, nucleosome disassembly is not merely a mechanical consequence of replication but likely an early regulatory step that constrains and shapes downstream inheritance outcomes**.** Understanding how nucleosome disassembly is controlled will be critical for defining how the initial conditions of histone recycling are established at the replication fork.

What mechanisms prevent histone loss during transit?

Once parental histones are released from DNA, they face a critical vulnerability: diffusion away from the replication fork would sever the link between histone modifications and their genomic context. The replisome mitigates this risk by providing multiple histone-binding platforms that transiently capture parental histones and retain them in close proximity to nascent DNA.

One attractive hypothesis is a relay mechanism whereby parental histones are sequentially transferred from histone-binding factors positioned near the helicase to those associated with sites of DNA synthesis [34]. For such a relay to be directional, downstream factors would need to exhibit higher effective affinity or more favorable kinetics for histone engagement. Whether such affinity hierarchies exist, or whether histone transfer is governed by spatial proximity and local concentration effects, remains unknown.

Importantly, histone binding by replisome components does not fully shield histones. Structural studies show FACT bound simultaneously to histones and Mcm2 [32,50], suggesting that chaperones such as FACT may remain associated with histones throughout transit. Consistent with this idea, mutations that disrupt FACT interaction with components of the replisome affect the proper inheritance of heterochromatin in fission yeast [34]. How parental histones are retained in a recycling-competent state during transit remains a central question for maintaining the link between chromatin information and genomic location.

How are parental histones released onto daughter DNA?

Another key decision point is the release of histones from replisome-associated binding sites and their redeposition onto newly synthesized DNA. Key questions regarding histone release remain unresolved. For example, is release triggered by local DNA synthesis, competition with newly synthesized histones, or conformational changes within replisome components as replication progresses?

This step ultimately determines whether parental histones reinforce their original chromatin domain or become misplaced, potentially seeding ectopic chromatin states elsewhere and compromising epigenetic fidelity. Failure to properly coordinate histone release and redeposition could have consequences. Premature release may increase the risk of histone loss or mislocalization, whereas delayed release could interfere with nucleosome assembly on nascent DNA, altering chromatin density or modification patterns. Thus, the timing and spatial control of histone redeposition represent a critical checkpoint in replication-coupled inheritance. Defining how histone release is timed and coordinated with DNA synthesis will be essential for understanding how positional fidelity is maintained during chromatin inheritance.

Does the incorporation of newly synthesized histones influence parental histone inheritance?

Following DNA replication, approximately half of the nucleosomes on each daughter strand are assembled from newly synthesized H3–H4. Deposition of new histones is also tightly coupled to replication through dedicated histone chaperones, most prominently CAF-1 and ASF1 [40,54]. In principle, these pathways could compete with the parental histone segregation machinery for binding sites on the replisome or for access to newly synthesized DNA.

Genetic evidence, however, suggests a hierarchy between parental and new histone pathways. Mutations in CAF-1 do not measurably affect parental histone distribution [61], whereas mutations in core parental histone recycling factors, such as Mcm2 or Dpb3/Dpb4, alter the distribution of newly synthesized histones [5–7,30]. These observations support a model in which parental histones are deposited first, with newly synthesized histones filling the nucleosomal gaps left behind.

However, single-molecule studies of DNA replication in *Xenopus* egg extracts suggest a different hierarchy. In this system, parental histones are predominantly evicted immediately after fork passage and are preferentially recycled only when the pool of newly synthesized histones is depleted, implying direct competition between parental and new histone deposition pathways [62]. These

results raise the possibility that the relative availability of new histones can actively shape inheritance outcomes, rather than simply buffering incomplete parental recycling. How parental and new histone deposition pathways are coordinated *in vivo*—whether through competition, temporal separation, or active prioritization—remains unresolved.

How is recycling balanced between daughter strands?

Parental histone inheritance is globally symmetric between leading and lagging strands. One explanation for symmetric inheritance is competition between strand-specific recycling pathways. Supporting this model, parental histone segregation becomes relatively symmetric when both leading- and lagging-strand pathways are simultaneously compromised [30,63]. However, DNA replication is intrinsically asymmetric. Leading-strand synthesis proceeds continuously, whereas lagging-strand synthesis is discontinuous and delayed by the need to generate sufficient single-stranded DNA for priming and Okazaki fragment initiation. Despite this fundamental asymmetry, parental histone inheritance is globally symmetric, indicating the existence of balancing mechanisms that compensate for differences in replication dynamics.

Replisome architecture may provide a balancing mechanism. Efficient coordination between leading- and lagging-strand synthesis requires physical coupling of polymerase activities, resulting in a folded replisome configuration. Cryo-EM structures place Pol α at the front side of the MCM (minichromosome maintenance) helicase, positioning the lagging-strand machinery close to sites of nucleosome disruption [22]. In this spatial arrangement, lagging-strand histone capture may occur earlier and more efficiently than predicted by linear models of fork progression.

An alternative, and not mutually exclusive, possibility is that histone deposition onto the two daughter strands is actively coordinated. In this view, parental histones may be redistributed in an alternating manner between leading and lagging strands, rather than independently captured by competing pathways. Consistent with this idea, disruption of one-strand-specific recycling pathway leads to a reduction in histone density without a corresponding increase on the opposite strand, arguing against simple competition [30]. Such coordination could arise from regulatory mechanisms within the replisome that enforce balanced distribution. Defining how symmetric histone inheritance is achieved despite asymmetric replication dynamics, and whether it reflects competition, coordination, or a combination of both, remains a key challenge.

How precisely do parental histones return?

Another important unresolved question in epigenetic inheritance is whether parental histones retain memory of their exact genomic positions following replication. Measurements of parental histone redeposition suggest that histones are typically returned within several hundred base pairs of their original locations [64–67,88]. Whether this reflects a fundamental biological limitation or the resolution limits of specific approaches remains unclear.

Replication-coupled histone recycling imposes unavoidable spatial constraints. If parental histones are released ahead of the replication fork and redeposited behind it, they will introduce a physical separation that depends on fork architecture and replication timing. This displacement is expected to be more pronounced on the lagging strand, where DNA synthesis is delayed relative to histone release. In the absence of retention mechanisms, immediate redeposition would, therefore, shift histone positions away from their original loci.

These considerations imply that positional memory requires a buffering or delay mechanism, in which parental histones are held in a protected state until the appropriate DNA substrate

becomes available. Replisome-associated histone-binding proteins and chaperones are well-positioned to provide such buffering, effectively decoupling histone release from redeposition. Whether histone retention time is actively regulated or passively constrained by replication kinetics remains unknown.

Importantly, absolute positional precision may not be required for functional inheritance [45]. Many chromatin states, particularly heterochromatin domains, are defined over kilobase-scale regions rather than at single-nucleosome resolution. In this context, approximate redeposition may be sufficient to preserve domain identity, especially when coupled with post-replicative feedback mechanisms that reinforce chromatin states. By contrast, regulatory elements such as promoters, enhancers, or boundary elements may be more sensitive to positional drift. Determining how different chromatin contexts tolerate, buffer, or correct positional imprecision will be essential for linking parental histone segregation to gene-specific regulatory outcomes.

Are all histones and marks equal?

Most mechanistic models of replication-coupled chromatin inheritance have focused on canonical histones, reflecting their central role in nucleosome stability and their tight coupling to DNA replication. However, chromatin is compositionally diverse, containing multiple histone variants and a wide array of PTMs [1,68]. Whether these different components are inherited through shared or distinct mechanisms remains largely unexplored.

Histone variants pose a particular conceptual challenge. Many of them are incorporated independently of DNA replication and rely on dedicated histone chaperones [68]. One might, therefore, expect variant inheritance to be uncoupled from replisome-mediated histone recycling. Surprisingly, strand-specific analyses reveal that the histone variant H2A.Z is distributed symmetrically between daughter strands during replication [52]. This observation suggests that at least some variants can engage replication-coupled inheritance pathways, either through direct capture by replisome-associated factors or through rapid, localized reincorporation following fork passage. Whether similar principles apply to other histone variants and how replication-independent and replication-coupled pathways are coordinated remain open questions.

An additional layer of complexity arises from the inheritance of histone modifications. Many replisome-associated histone-binding proteins, including Mcm2, interact primarily with the histone core rather than the flexible tails that carry most PTMs; yet, strand-specific mapping of modified histones reveals unexpected biases of H3K9me3 [69]. It shows a reproducible leading-strand bias in wild-type cells, raising the possibility that modified histones may be selectively stabilized, retained, or redeposited through interactions with modification-specific readers. Such selectivity could be mediated by chromatin-associated effector complexes that bridge histone marks to the replication machinery. In mammals, the HUSH (human silencing hub) complex, which binds H3K9me3, associates with polymerase ε, suggesting a potential mechanism by which H3K9me3-marked nucleosomes could be preferentially handled during replication [69]. More broadly, these observations raise the possibility that epigenetic inheritance operates through a hierarchy of mechanisms, in which certain marks or variants receive additional layers of regulation to ensure their faithful transmission.

Together, these findings challenge the notion of a single, universal mechanism of epigenetic inheritance. Instead, they point to mark- and variant-specific strategies, tuned to the functional demands of different chromatin states.

How are weak interactions converted into a robust read–write mechanism?

Histone PTM-mediated recruitment of modifying enzymes is central to the classical read–write cycle that restores chromatin states after DNA replication [2]. However, this model faces a fundamental biophysical challenge: the binding affinities of most PTM reader domains for individual histone marks are relatively weak. How can such low-affinity interactions efficiently recruit and retain histone-modifying enzymes at specific genomic loci, particularly in the context of chromatin dilution following replication?

One emerging solution is that histone modifications rarely act in isolation. Instead, they are frequently embedded within networks of modification crosstalk that amplify enzymatic activity and stabilize chromatin association. A recurring theme is the involvement of histone monoubiquitination in stimulating methyltransferase function [70]. For example, H3K14 ubiquitination enhances the catalytic activity of the H3K9 methyltransferases Clr4/SUV39 [71–75,89,90]; H2AK119 ubiquitination promotes PRC2-mediated H3K27 methylation [76,77]; and H2BK120 ubiquitination stimulates both H3K4 and H3K79 methyltransferases [78–81]. In particular, H3K14ub and H3K9me3 form a feedback loop to promote heterochromatin spreading and inheritance [71]. In these cases, one modification does not merely recruit an enzyme but directly enhances its activity, effectively converting weak recognition events into robust chromatin modification.

Such stimulation has important functional consequences. By integrating low-affinity interactions into a cooperative network, they can generate bistable chromatin states that are resistant to stochastic fluctuations. These systems are relatively insensitive to small perturbations in histone mark density but respond sharply once a threshold is crossed, enabling reliable maintenance of chromatin states while suppressing noise introduced during DNA replication. How these cooperative circuits are integrated with replication fork dynamics remains an important unresolved question for understanding how chromatin states are both faithfully inherited and dynamically regulated.

How is chromatin-based inheritance regulated?

Over the past decade, major advances have identified the core factors and molecular interactions that mediate parental histone segregation during DNA replication. These studies have largely treated histone recycling as a constitutive process embedded within the replication machinery. An important and largely unexplored question, however, is whether and how these pathways are dynamically regulated in response to cellular state, environmental cues, or developmental context.

One axis of regulation may arise from replication stress or altered replication dynamics. Perturbations such as fork slowing, stalling, or collapse change the timing and spatial organization of replisome components and could directly affect histone capture, retention, and redeposition. Whether histone-binding activities, chaperone interactions, or release kinetics are modulated under these conditions, and how such modulation influences epigenetic stability, remains largely unknown.

A second, and perhaps more provocative, question concerns developmental regulation and asymmetric cell fate decisions. Direct evidence for regulated histone inheritance in development remains limited. Nevertheless, in stem cell divisions, daughter cells frequently adopt distinct transcriptional programs despite inheriting identical DNA sequences, raising the possibility that parental histone segregation could be regulated to favor the asymmetric inheritance of chromatin states. Studies in germline stem cells and intestinal stem cells have reported preferential

segregation of parental histones to the stem cell daughter [82,83], which has been attributed to the regulation of the replication machinery [84–86]. However, the interpretation and reproducibility of these findings have recently been challenged [87]. Therefore, whether asymmetric cell fate specification is regulated by active reprogramming of the histone segregation machinery remains an open question.

Concluding remarks

The inheritance of chromatin states through DNA replication is no longer viewed as a passive consequence of histone mark copying but as an active and coordinated process centered at the replication fork. Work summarized in this review demonstrates that parental histone segregation is mediated by replisome-coupled pathways that dismantle, capture, retain, and redeploy histones in close coordination with DNA synthesis.

As in the past, continued technical advances are likely to refine and extend current models. Integrating quantitative approaches with high-resolution, locus-specific measurements will be essential for defining the kinetics and constraints of parental histone segregation. In parallel, *in vitro* reconstitution of chromatin replication will enable structural and single-molecule analyses that provide more precise mechanistic insights. Finally, extending these findings from model systems to multicellular and developmental contexts will further elucidate how epigenetic memory is maintained and reshaped across cell divisions, reinforcing the replication fork as a central nexus linking DNA replication and chromatin inheritance.

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Declaration of interests

The author declares no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used ChatGPT to improve language and readability. After using this tool/service, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Outstanding questions

What is the unit of inheritance at the replication fork?

Do parental histones transmit epigenetic information exclusively as intact H3-H4 tetramers, or can partially disassembled intermediates, such as hexamers, also function as inheritance units?

How are nucleosomes dismantled ahead of the replication fork?

What molecular mechanisms coordinate progressive nucleosome disassembly with histone retention?

How are parental histones captured and retained during transit?

How do multiple replisome-associated histone-binding factors cooperate to retain parental histones near the fork, and what determines the directionality and efficiency of histone transfer?

How are parental histones released and redeposited onto daughter DNA?

What signals or structural transitions trigger histone release from replisome-associated factors?

How do newly synthesized histones interact with parental histone recycling pathways?

Do parental and new histone deposition pathways compete or cooperate during replication, and how is their balance regulated *in vivo*?

How is symmetric inheritance achieved despite asymmetric DNA replication?

What mechanisms compensate for differences between leading- and lagging-strand synthesis to ensure balanced parental histone segregation?

How precise is positional memory after replication?

To what extent do parental histones return to their original genomic locations, and how much positional imprecision can be tolerated by different chromatin states?

Are all histones and histone marks inherited equivalently?

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Do specific histone variants or modifications engage distinct inheritance pathways that bias their retention, segregation, or restoration?

How do feedback loops stabilize chromatin states after replication?

What quantitative thresholds and kinetic parameters allow feedback-mediated reinforcement to convert partial inheritance into stable epigenetic memory?

Can parental histone segregation be regulated?

Are histone segregation pathways modulated by replication stress, signaling pathways, or developmental context to enable adaptive or asymmetric inheritance?

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