# Replicating chromatin in the nucleus: A histone variant perspective

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Current Opinion in Cell Biology

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**Abstract**

In eukaryotes, chromatin and DNA replication are intimately linked, whereby chromatin impacts DNA replication control while genome duplication involves recovery of chromatin organisation. Here, we review recent advances in this area using a histone variant lens. We highlight how nucleosomal features interplay with origin definition and how the order of origin firing links with chromatin states in early mammalian development. We next discuss histone recycling and *de novo* deposition at the fork to finally open on the post-replicative recovery of the chromatin landscape to promote maintenance of cell identity.

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**Current Opinion in Cell Biology** 2024, **89**:102397

This review comes from a themed issue on **Cell Nucleus (2024)**

Edited by **Evi Soutoglou** and **Noriko Saitoh**

For complete overview of the section, please refer the article collection - Cell Nucleus (2024)

Available online 8 July 2024

**https://doi.org/10.1016/j.ceb.2024.102397**



## Introduction

Every cell division, for two daughter cells to inherit equivalent genetic information, the genome must be fully and accurately duplicated in S phase. In mammals, this involves the use of about 30,000−50,000 replication initiation sites, of which only a subset fires stochastically in S phase. However, how these sites are defined and selected to fire has remained a puzzle. In G1, origins are licensed by the binding of the origin recognition complex (ORC), followed by the loading of the mini-chromosome maintenance (MCM)2−7 helicase. The origins with the highest probability of usage represent the most efficient ones, which initiate in early S phase, while less efficient origins fire later. Thus, a spatiotemporal order of firing, known as the replication timing (RT) programme is set in place in a cell type-specific manner. The issue of origin selection, involving ORC binding, origin licensing and firing, which are central for DNA replication, has been recently reviewed [1]. The importance of the regulation of the RT programme throughout development and differentiation has increasingly gained interest emphasizing the tight correlation between chromatin features, origins, and replication timing [2]. This is an intricate question because while chromatin features may impact DNA replication, in turn chromatin replication represents a challenge for the maintenance of both genomic and epigenomic integrity at each cell division in each cell lineage. Indeed, in addition to replicating DNA, its organization in chromatin is also duplicated. Ensuring a full nucleosome complement on newly synthesized DNA requires the concerted recycling of parental and deposition of *de novo* synthesised histones [3]. This mixing with new histones dilutes parental histones and their associated marks. To restore pre-existing post-translational modifications (PTMs) on chromatin, read-write mechanisms have been proposed to mark new histones based on information from the old ones [4]. Thus, the mechanisms regulating DNA replication interplay with the recovery of epigenetic states which can itself impact DNA replication. Here, we will discuss recent findings linking DNA replication and chromatin from the perspective of an underappreciated component of chromatin: histone variants. We will focus on four main aspects: (i) how ORC binding interplays with nucleosome organisation/composition, (ii) how the RT programme connects with the reshaping of chromatin in mammalian embryos, (iii) how chromatin dynamics coordinates with the replication fork progression and (iv) how this contributes to maintain chromatin integrity, cell state and function.

## How are origin positions and efficiency defined?

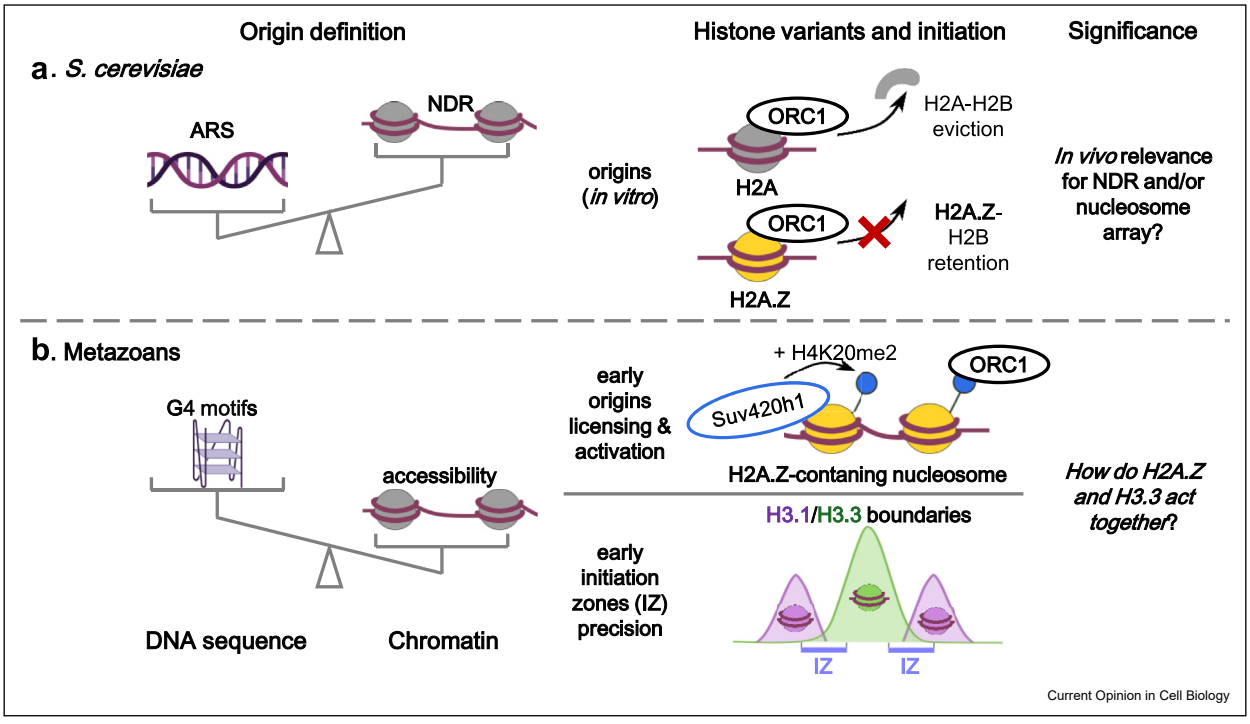
### Nucleosome organisation at origins: interplay with ORC binding and histone variants

In *Saccharomyces cerevisiae*, initiation sites rely on specific DNA sequences, known as the autonomous replicating sequences (ARS). They are recognised by ORC (composed of 6 members, ORC1-6) as a first step in the process of initiation. Although ∼30% origins lack the

ARS consensus sequence, all exhibit a nucleosome-depleted region (NDR) flanked by well-positioned nucleosomes [5]. Furthermore, ORC1 binding is necessary and sufficient to establish these nucleosome arrays *in vivo* and on *in vitro* reconstituted chromatin in the presence of remodellers [5]. Following these first observations, recent work further hints to the importance of chromatin for replication initiation. Firstly, on an ARS-containing plasmid *in vitro*, *S. cerevisiae* ORC1 colocalised with nucleosomes regardless of the underlying sequence [6]. Thus, even in budding yeast, nucleosome organisation at the origins may contribute to ORC association. Secondly, ORC1 mutants impaired in their ability to establish nucleosome arrays lead to defects in S phase entry and reduced cell viability [7]. Finally, *in vitro* ORC1 directly remodels nucleosomes containing H2A-H2B but not H2A.Z-H2B dimers [8]. While the *in vivo* functional significance of this property remains to be explored, it is tempting to speculate that it could contribute to define the NDR region and/or nucleosome array at origins. Collectively, these data imply that, even at *S. cerevisiae* origins, distinct dynamics between nucleosome and ORC binding are at play (Figure 1a).

In metazoans, origins are not sequence-defined but rely on both DNA- and chromatin-based information [9]. More specifically, they often contain regions that can form G quadruplexes (G4) and show chromatin accessibility. In mammalian cells, it has been challenging to obtain a consensus mapping of origins at high resolution [1,10]. Instead, initiation sites within initiation zones (IZs) of 20 up to ~150 kb in length may be used stochastically. Firing efficiency also correlates with transcription, presence of "active" histone PTMs and open chromatin [1]. In avian DT40 cells, dimeric G4 elements spaced 250 bp apart can establish a NDR followed by a nucleosome array at the $\beta^A$-globin origin, promoting early initiation [11]. Thus, modulating nucleosome organisation via genetic features may be a common mechanism across species to regulate origin

**Figure 1**




**Origin definition: balance between sequence and chromatin-based information**

**a**. In budding yeast, origins are defined mainly based on sequence. Replication initiation starts at the autonomous-replicating sequence (ARS), which is recognised by ORC. Budding yeast origins also have a characteristic nucleosome organisation comprising an NDR over the ARS flanked by a nucleosomal array. The presence of an NDR and a flanking nucleosome is important for origin function and promote ORC1 binding independently of DNA sequence. The ARS may also contribute to establish the NDR due to its sequence properties which impede nucleosome formation. *In vitro, S. cerevisiae* ORC1 shows variant-specific eviction activity for H2A-H2B, but not H2A.Z-H2B, indicating a possible link between histone variants and origins in yeast.
**b**. In metazoans, chromatin features play a larger role than DNA sequence in origin definition. Initiation sites are enriched in G-rich elements like G4 motifs, which may preclude nucleosome formation and promote origin accessibility. Mammalian ORC1 does not exhibit sequence-specific binding but can recognise the H4K20me2 mark. At early origins, H2A.Z can stimulate licensing by recruitment of Suv420h1, which deposits H4K20me2. At a larger scale, H3.3/H3.1 boundaries established by the H3.3-specific chaperone HIRA, define early initiation zones (IZs).
Variant-containing nucleosomes are labelled in yellow (H2A.Z), purple, (H3.1) and green (H3.3).

activity. Notably, mammalian ORC1 recognises nucleosomes harbouring the H4K20me2 modification, imposed by the Suv420h1 enzyme [12]. Since the histone variant H2A.Z can directly recruit this enzyme at origins, this can potentially link chromatin state to licensing and activation of origins [13]. Additionally, H2A.Z and the heterochromatin mark H3K27me3 were also recently found enriched at efficient IZs in human cells [14]. Indeed, ORC1 has been reported to associate with H3K9me3, H3K27me3 and H4K20me3 nucleosomes [15]. However, as H2A-H2B dimers are more labile than H3−H4 tetramers [16], how H2A.Z may act as a heritable mark for origins is intriguing. Notably, the post-replicative recovery of H3K27me3 on newly synthesized H3−H4 has been linked to local H2A-H2B recycling [17] (discussed below). Thus, a closer examination of H2A.Z recycling and H4K20me2 restoration is needed, including considering whether metazoan ORC can handle H2A-H2B in a variant-specific manner. This highlights the importance of chromatin and potentially distinct histone variants for replication initiation in mammals (Figure 1b).

#### New ways of defining early initiation zones: H3.3/H3.1 balance

In mammalian cells, early-replicating domains show an enrichment in the major non-replicative histone H3 variant, H3.3, while late replicating/heterochromatin regions rather show enrichment in the replicative H3.1 [18]. The expression and deposition of these two variants display distinct features (see Delaney et al., 2023 [19] for review). H3.3 expression is not cell cycle regulated. Its DNA synthesis-independent (DSI) deposition is mediated by the histone regulator A (HIRA) complex at sites of active transcription and high nucleosome turnover. In contrast, the expression of replicative H3.1 peaks in S phase. Its deposition occurs in a DNA synthesis-coupled (DSC) manner relying on the chromatin assembly factor 1 (CAF-1) complex. To explore how these distinct H3 variant dynamics interplay to establish the genome wide H3.3/H3.1 enrichment patterns, Gatto et al. (2022) followed their *de novo* deposition during S phase [20]. They revealed that the dual deposition mode of the variants leads to the formation of boundaries at discrete H3.3-enriched sites flanked by H3.1 genome-wide. These boundaries corresponded to early replication IZs. While the variant distribution with respect to early IZs could merely reflect their deposition dynamics, the fact that knock out (KO) of the H3.3 chaperone HIRA impacted replication underlined a potential role in regulating early IZs in mammals [20] (Figure 1b). Indeed, the absence of HIRA led to two classes of effects on early-replicating sites. First, at boundaries associated with transcription, the patterns of both H3.3 and nascent DNA synthesis became less well-defined, therefore early initiation was termed blurred. Second, at H3.3/H3.1 boundaries with low or no transcriptional activity, a complete loss of both H3.3 enrichment and nascent DNA synthesis occurred; hence these early initiation zones became buried. Thus, HIRA proved critical for a chromatin-based definition of early IZs to ensure precision in initiation in transcribed regions and allow initiation in non-transcribed regions [20].

HIRA-mediated H3.3 targeting may impact early IZs by acting on either licensing or firing of origins, or both. First, since lack of HIRA leads to an increased sensitivity to DNase I digestion [21], a reduction in nucleosome density and positioning may occur, affecting ORC binding. Second, H2A.Z, a hallmark of unstable nucleosomes with H3.3 [22], is present at H3.3/H3.1 boundaries [20]. Thus, at these sites the two variants may act in concert to promote firing efficiency. Finally, H3.3 displays a unique serine residue at position 31, which can be reversibly phosphorylated [19]. This modification may provide a unique mechanism to attract or repel licensing or firing factors in a dynamically regulated manner.

## Replication timing programme: controlling the pattern of origin firing

#### RT programme remodelling along with chromatin state re-establishment in early mammalian development

In S phase, origins are activated sequentially according to the cell type-specific RT programme. RT leads to consistent patterns of regions that replicate in early or late S phase within a cell type. In general, RT correlates with transcription, chromatin states and 3D genome organisation across cell types with few exceptions in embryonic stem cell (ESC) differentiation [2]. Given that zygotes and 2-cell stage embryos show distinct transcriptional status [23] and 3D chromatin architecture [24] exploring their interconnections with RT proved attractive to study. In mouse, from zygotes to 2-cell stage embryo and onward, RT starts as a rather variable programme which acquires an increasingly clearer partitioning in subsequent developmental stages [25]. These properties have been confirmed in mouse [26] and extended to bovine embryos [27]. RT changes at the 2-cell stage in mouse have been linked to chromatin accessibility but can occur independently of the active transcription programme [25]. This time also coincides with the time of establishment of canonical H3.3 pattern [28]. Thus, given the role of H3.3/H3.1 boundaries in defining early IZs in somatic cells [20], examining these boundaries in early development would be important. Interestingly, H3.3 enrichment increases along with advanced (earlier) RT of the repetitive element MERVL upon induction of 2 cell-like cells from mouse ESCs in culture [29]. Furthermore, during naïve to primed mouse ESC differentiation, H3.3 enrichment and changes are the best predictors of steady-state RT

and RT shifts, respectively [26,30]. Thus, these data underline a possible link between usage of the H3.3 variant and early replication during early development transitions.

## Coordination of histone dynamics at the replication fork: key to maintain epigenetic state

Replication itself poses a challenge to the maintenance of origin and RT memory if they are chromatin based. During S phase, parental nucleosomes with their specific histone variant and PTM composition undergo a two-fold dilution in the wake of the replication fork. The current view poses a tight coordination of *de novo* histone deposition and parental histone recycling (Figure 2) along with reader–writer complexes to re-establish the pre-existing marks on chromatin prior to the next S phase [19,31].

### Deposition of newly synthesized histones in S phase

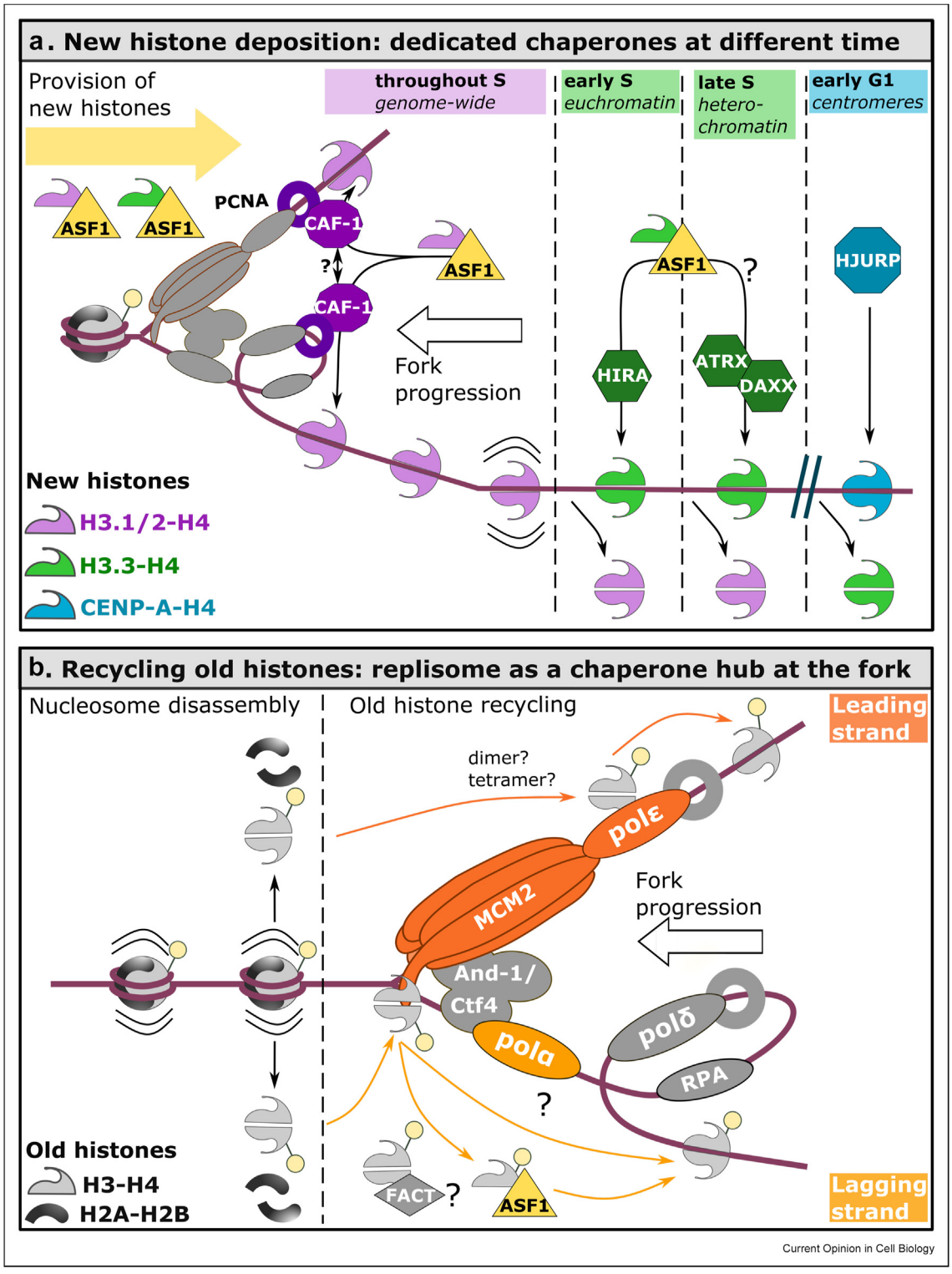
The CAF-1-dependent DSC deposition of *de novo* synthesised replicative H3.1-H4 (Figure 2a) plays a crucial role in maintaining genome and epigenome integrity. This pathway can ensure restoration of a proper nucleosome density, and/or also establish a link with heterochromatin establishment by exploiting the interaction of CAF-1 with HP1a-SetDB1 [32] or the enzymatic subunit EZH2 of Polycomb repressive complex 2 (PRC2) [33] (Table 1). In *S. cerevisiae* which does not have the repressive H3K9me3 or H3K27me3 marks, CAF-1 contribution to maintain inactive states most likely relates to its chromatin assembly properties ensuring nucleosome occupancy [34]. In mouse ESCs, by inducing rapid protein depletion of CAF-1 in S phase, heterochromatin accessibility increased [35]. In addition, transcription increased in H3K27me3 heterochromatin domains, potentially resulting from dilution of the mark or defects in chromatin assembly leading to lower nucleosome density [35]. Deposition of new histones can also occur in a DSI manner, uncoupled from fork progression [3] (Figure 2a). For this reason, DSI incorporation in S phase has largely been overlooked. However, DSI deposition of the non-replicative H3.3 by HIRA in S phase is crucial to define early IZs and to rapidly recover H3.3 in the wake of replication [20] (discussed above). Furthermore, in the absence of CAF-1, incorporation of H3.3 by HIRA can act as a compensatory mechanism [21]. This is in line with the recent observation that following CAF-1 depletion, nucleosome density is increased in mature compared to nascent chromatin in early-replicating regions, implying nucleosomes are assembled there in a DSI manner in the hours after fork passage [35]. Finally, in heterochromatin another H3.3-specific chaperone, death domain-associated protein (DAXX), interacts with the methyltransferases SetDB1 and Suv39h1 and promotes K9 methylation of new H3.3 prior to chromatin incorporation [36] (Table 1). DAXX targets H3.3 to repetitive and transposable elements, promoting the presence of H3.3K9me3 which is critical for their repression in mouse ESCs [19]. These studies highlight an underappreciated role for DSI deposition in supporting both active and inactive chromatin state maintenance in S phase by targeted delivery of histone variants with distinct pre-deposition marks by their chaperones.

### Read-write mechanisms and recycling of parental histones in S phase

Parental histone recycling on the leading and lagging strands involves distinct sets of chaperones, many of which are replisome components (Figure 2b). On the leading strand, two non-essential subunits of DNA polymerase ε (Polε), POLE3-4 (dpb3-4 in yeast), handle H3–H4 tetramers [37,38]. On the lagging strand, the MCM2 [39,40] subunit of the replicative helicase cooperates with the H3–H4 chaperone anti-silencing function 1 (ASF1) [41] and DNA polymerase α (Polα, in yeast and mammals) [42,43], as well as chromosome transmission fidelity 4 (Ctf4, in yeast) [42] to recycle parental H3–H4. In yeast, a non-essential subunit of the lagging strand polymerase δ (Polδ), Pol32, was also recently shown to contribute to H3–H4 recycling on this strand [44,45]. In mammals, parental H2A-H2B is also transferred on the lagging strand by Polα, while its recycling mechanism on the leading strand remains unclear [17] (for review see Flury and Groth, 2024 [46]). It would be interesting to investigate if other replisome-associated factors could contribute to histone recycling and how this may be impacted by replication stress. Symmetric local recycling is required to maintain the epigenetic landscape without affecting overall nucleosome occupancy [31]. Curiously, combined loss of MCM2 histone binding and dpb4 (mcm2-2A dpb4Δ) attenuates both the strand bias and the impaired silencing compared to mcm2-2A single mutant alone in fission yeast. This highlights the importance of symmetry even in a context where less parental histones are recycled [47].

Even when histones are efficiently recycled locally, parental PTMs are usually diluted by half after replication, yet restored prior to the next S phase. Read-write mechanisms re-establishing repressive marks have been linked to *de novo* histone deposition (as discussed above), but they also require histone recycling for templating (Table 1). Concerning H3K27me3, association of the general histone chaperone nucleophosmin (NPM1) with MCM2 specifically in late S phase has been invoked to ensure H3.1K27me3 maintenance and repression of PRC targets [48]. Additionally, the local recycling of H2A-H2B dimers by Polα allows the formation of nucleosomes with 'old' H2A-H2B and 'new' H3–H4 [17]. Since PRC2 can recognise the parental

**Figure 2**




**Histone dynamics during replication**

**a**. *De novo* deposition of histones mostly in S phase involves dedicated histone chaperones at distinct locations and time. Newly synthesized or naïve H3.1/2-H4 and H3.3-H4 dimers harbor pre-deposition marks. Their flow under the control of ASF1 allows a transfer to variant-specific chaperones. The CAF-1 complex promotes new H3.1/2-H4 deposition coupled to fork progression through interaction with PCNA on both nascent DNA strands, a process that occurs genome-wide. The formation of H3.1-H4 tetramers requires dimerisation of p150, the largest CAF-1 subunit. This dimerization may contribute to coordinate *de novo* H3.1/2-H4 deposition leading and lagging strand. In early S, in euchromatin HIRA rapidly replaces H3.1/2-H4 with newly

H2AK119ub and catalyse H3K27me3 on new histones, this can provide a short-term memory of Polycomb-mediated repression. Concerning H3K9me3, unlike most PTMs which are almost symmetrically recycled (Stewart−Morgan et al., 2020 [31]), it shows a strong bias towards the leading strand in mid-S, which is undetectable in G1 [49]. The strongest H3K9me3 asymmetry is detected at late IZs enriched in L1 LINE elements oriented head-on (HO, i.e. contrary) to replication fork direction. This H3K9me3 bias depends on both the transcription−driven association of the human silencing hub (HUSH) complex to HO L1 LINEs and its interaction with Polε subunits [49]. This H3K9me3 asymmetry, possibly promoted by HUSH passing H3K9me3-H4 to Polε, may also be affected by transcription. However, such bias is not detected for the repressive H4K20me3 mark, which may co-occur along with H3K9me3 and should be recycled together [4]. Thus, another interpretation would be that the interaction of Polε with HUSH ensures its availability specifically at expressed HO L1 LINEs. Finally, since a bias for H3.3 has been reported towards the leading strand in wild-type (WT) cells [50] and DAXX-mediated H3.3K9me3 deposition is important for repeat silencing, how a variant bias could play a role in this context will be interesting to explore.

## Coordinating *de novo* deposition and recycling at the fork

Coordination of parental histone recycling and deposition of newly synthesized histones at the fork ensures efficient chromatinization of nascent DNA. This requires a proper control of the supply of new and parental histones involving the general H3−H4 chaperone ASF1. In addition to the importance of ASF1 in histone recycling, *de novo* deposition by both DSC and DSI pathways take advantage of ASF1 interaction with CAF-1 and HIRA to provide new histones [3] (Figure 2). Furthermore, disrupting the balance between the levels of histones and ASF1 impairs DNA unwinding, highlighting the importance of coordination between DNA replication and histone dynamics at the fork [41]. The importance of ASF1 for recycling may also have a variant-specific aspect. Upon ASF1 depletion, local H3.3 recycling at replication sites is disrupted only in mid/late, but not early S [18]. Thus, at early IZs, HIRA may coordinate *de novo* deposition [20] and parental H3.3 recycling, as reported in the context of transcription [51]. Interestingly, the structure of the HIRA trimer is comparable to that of the yeast recycling factor Ctf4 [52]. Finally, recent work showed that ASF1 not only promotes histone protein provision but also contributes to regulate replicative histone mRNA metabolism [53]. This further emphasizes the importance of ASF1 in acting as a 'chaperone checkpoint' to balance the new and parental histone supply at the replication fork.

In symmetric recycling mutants [31,54], new histone deposition also shows a strand bias indicating *de novo* deposition and recycling are coordinated on each strand. The sliding clamp proliferating cell nuclear antigen (PCNA), present on each strand as a trimer, could act as a coordination hub for polymerases and chromatin-related proteins [55]. For example, PCNA contacts CAF-1 and the leading strand polymerase Polε through the same motif [56], resulting in a mutually exclusive interaction [57]. In contrast, *S. cerevisiae* PCNA interacts with CAF-1 and the lagging strand polymerase Polδ through different surfaces [44]. This may play a role to balance recycling by replisome components and new histone deposition by CAF-1. Further studies using different interaction mutants will help to dissect these mechanisms.

## Chromatin dynamics during replication: importance of symmetric recycling for cell state

The *in vivo* importance of symmetric recycling was recently illustrated in mouse ESCs. MCM2-2A histone binding mutants compromised in symmetric recycling showed reduced pluripotency and differentiation potential [50,54,58]. These cells exhibit an asymmetric H3K27me3 enrichment on the two strands in S, which is maintained in the following G1 and results in a genome-wide redistribution of the mark. This impacts H3K27me3 at the promoters of bivalent genes with a transcriptional dysregulation in steady state [54] and differentiation [50]. H3K9me3 is also redistributed, showing a reduction at repetitive elements linked to their transcriptional upregulation [50,58]. Interestingly, in cultured MCF7 cancer cells the MCM2-2A mutation gave rise to similar results, reducing H3K27me3 at genes linked to development and differentiation without affecting proliferation rates [59]. Yet, after

synthesized H3.3-H4. In late S, at heterochromatin domains, ATRX/DAXX replaces H3.1/2-H4 with H3.3-H4. Only later during the following G1 at centromeres, HJURP ensures the new deposition of CENP-A-H4 replacing placeholders H3.3-H4 deposited in S. For reviews, see Ray-Gallet and Almouzni (2021) [3], Delaney et al. (2023) [19].

**b**. Ahead of the replication fork, nucleosomes are disassembled into an H3−H4 tetramer and two H2A-H2B dimers. Their recycling onto newly synthesized DNA is largely mediated by replisome components, which do not discriminate between the H3 variants. POLE3-4 (non-essential Polε subunits) handle H3−H4 on the leading strand. On the lagging strand, H3−H4 recycling involves MCM2 which may transfer histones onto DNA directly or through interaction with ASF1. Other replisome components like Polα also contribute to lagging strand recycling in mammalian cells, although it is not clear how they receive and hand over histones. Additional aspects not shown in this figure for simplicity include (i) HJURP which together with MCM2 contributes to CENP-A-H4 recycling on both strands at centromeres in mid/late S [62], (ii) Polα for H2A-H2B recycling on the lagging strand [17] and (iii) possible roles for FACT [63–65], AND-1/Ctf4 [42], Polδ [44,45] and RPA [66] for recycling on the lagging strand as demonstrated in yeast. For review, see Flury and Groth (2024) [46].

**Table 1**

**Chromatin state recovery links to *de novo* deposition and recycling factors at the fork.**

| | Histone chaperone | Variant specificity | Strand specificity | Promote recovery of | Through interaction with | Location/time | Organism | References |
|---|---|---|---|---|---|---|---|---|
| ***De novo* deposition** | **CAF-1** | **H3.1-H4** | both | **H3.1** | PCNA | genome-wide | mouse, human | [56,67,68] |
| | | | | **H3K9me3** | HP1-SetDB1 | heterochromatin/late S | | [32,33] |
| | | | | **H3K27me3** | EZH2 | | | |
| | **HIRA** | **H3.3-H4** | N.D. | **H3.3** | *DNA?* | early replication | mouse, human | [20,21] |
| | | | | **H3K27me3 accessibility?** | Polycomb (EZH2, SUZ12, Jarid2) SWI/SNF | N.D. | | [69,70] |
| | **DAXX** | **H3.3-H4** | N.D. | **H3.3** | HP1-SetDB1, Suv39h1 | heterochromatin | mouse, human | [36,71] |
| | | | | **H3K9me3** | | N.D. | | |
| | **HJURP** | **CENP-A-H4** | N.D. | **CENP-A** | CENP-C | centromeres/early G1 | mouse, human | [72] |
| **Recycling** | *Bone fide histone chaperones* | | | | | | | |
| | **ASF1** | **H3.1/H3.3-H4** | N.D. | **H3.1, H3.3** | MCM2 | genome-wide | human | [41] |
| | | | | **H3.1** | N. D. | late S | | [18] |
| | | | | **H3.3** | | early & late S | | |
| | **FACT** | **H2A-H2B, H3–H4** | | | N.D. | genome-wide | yeast | [63–65] |
| | **HJURP** | **CENP-A-H4** | N.D. | **CENP-A** | N.D. | centromeres | human | [62] |
| | ***HIRA?*** | ***H3.3-H4*** | *N.D.* | ***H3.3*** | *N.D.* | *early replication* | *human* | [51] |
| | *Replisome components chaperoning histones* | | | | | | | |
| | **Polε (POLE3/4)** | **H3.1/H3.3-H4** | leading | **H3.1, H3.3** | N.A. | genome-wide | yeast, human | [37,38] |
| | | | | **H3K9me3** | HUSH complex | L1 LINEs/mid-S onward | mouse | [49] |
| | **MCM2** | **H3.1/H3.3/CENP-A-H4** | lagging | **H3.1, H3.3, CENP-A** | N.A. | genome-wide | yeast, mouse, human | [39–42] |
| | | | | **H3.1K27me3** | NPM1 | heterochromatin/late S | mouse | [48] |
| | **Polα** | **H3.1/H3.3-H4** | lagging | **H3.1, H3.3** | N.A. | genome-wide | yeast, mouse | [42,43] |
| | | **H2A-H2B** | lagging | **H3K27me3** | Polycomb | N.D. | mouse | [17] |
| | | ***H2A.Z-H2B?*** | *lagging* | ***H4K20me2*** | *Suv420h1?* | *N.D.* | *human* | [13] |
| | **Polδ (Pol32)** | **H3–H4** | lagging | N.D. | PCNA | genome-wide | yeast | [44,45] |
| | **CTF4 (AND-1)** | **H3–H4** | lagging | N.D. | MCM2, Polα | genome-wide | yeast | [42] |
| | **RPA** | **H3–H4** | | N.D. | | genome-wide | yeast | [66] |

Rows in italics and question marks indicate speculative functions. HJURP, Holliday junction recognition protein; FACT, facilitates chromatin transcription; AND-1 (acidic nucleoplasmic DNA-binding protein); RPA, replication protein A; CENP-A, Centromeric protein A; EZH2, Enhancer of zeste-2; SUZ12, Suppressor of zeste-12; SWI/SNF, Switch/sucrose nonfermenting; N.D. – not determined. N.A. – not applicable.

transplantation in mice for 4 or 7 weeks, these cells showed more growth, higher metastatic potential and larger cell heterogeneity compared to WT MCF7 cells [59]. Thus, symmetric histone recycling could ensure maintenance of the H3K27me3 landscape and thereby cell state [50,54,58].

## Conclusions and perspectives

The balance of information from DNA sequence versus chromatin should be considered to define origin across species. To understand how chromatin contributes to the regulation of replication initiation in S phase while it is itself disrupted by the passage of the fork, it is critical to consider how nucleosome features associated with origins are recovered and transmitted post-replication. One novel aspect in the heritable nature of origin definition will be to integrate the contribution of histone variants with their distinct deposition modes and dynamics. A mechanistic understanding will require integrating ORC1 binding and its chromatin remodelling activity on combinations of metazoan sequences with different nucleosome composition. As ORC1 reassociates with chromatin after mitosis, the kinetics of chromatin state recovery together with G1 length may contribute to the regulation of this process.

Notably, replication organisation changes both during development and in the context of cancer. In early embryogenesis, RT shows distinct features, whereas during stem cell differentiation major replication changes correlate with H3.3 redistribution. Thus, it will be important to determine if H3.3 contributes to reshaping replication in this context. In cancer, a driver role has been attributed to missense mutations in both H3.1 and H3.3, termed 'oncohistones'. These substitutions have been documented to impact global histone PTM patterns and levels in a variant-specific fashion [60]. H3.3K27M specifically blocks cell reprogramming and enhances growth during *Drosophila* development, phenocopying Polycomb suppression [61]. Similarly, recycling mutants confer a proliferative advantage to cancer cells *in vivo*, accompanied by reorganisation of PTM distribution and dysregulation of developmental genes. However, in both cases, it is still unknown if they also interfere with the replication programme of the cells. Future investigations dissecting the impact of chromatin state recovery on replication could reveal if it may constitute a therapeutic vulnerability in the context of cancer. Finally, the processes discussed above all take place in the context of higher-order chromatin architecture in the nuclear environment, which may be both instructive and affected by replication (rev in Oji et al., this issue) and histone variant incorporation. We envision that a holistic approach that integrates the different aspects of regulation and mechanics of replication is a way forward to understanding how chromatin state is propagated alongside genome doubling.

## CRediT author statement

Tina Karagyozova: Conceptualization, Writing original draft, Writing – review & editing.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgements

We thank Dominique Ray-Gallet and Jean-Pierre Quivy for critical reading of the manuscript. We apologize to all those whose work we were unable to refer in this review due to space constraints. This work was supported by the European Research Council (ERC-2015-ADG-694694 "ChromADICT"), the Ligue Nationale contre le Cancer (Equipe labellisée Ligue), France and Agence Nationale de la Recherche, France (ANR-11-LABX-0044_DEEP, ANR-10-IDEX-0001-02 PSL, and ANR21-CE-11-0027 "CAFinDs"). T. K. was supported by individual funding from H2020 MSCA-ITN – ChromDesign (Grant No. 813327) and La Ligue Nationale contre le Cancer (Grant No. TDLM23697).

## References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

1. Hu Y, Stillman B: **Origins of DNA replication in eukaryotes**. *Mol Cell* 2023, S1097276522012035, https://doi.org/10.1016/j.molcel.2022.12.024.

2. Vouzas AE, Gilbert DM: **Replication timing and transcriptional control: beyond cause and effect — part IV**. *Curr Opin Genet Dev* 2023, **79**, 102031, https://doi.org/10.1016/j.gde.2023.102031.

3. Ray-Gallet D, Almouzni G: **The histone H3 family and its deposition pathways**. In *Histone mutations and cancer advances in experimental medicine and biology*. Edited by Fang D, Han J, Springer; 2021:17–42, https://doi.org/10.1007/978-981-15-8104-5_2.

4. Escobar TM, Loyola A, Reinberg D: **Parental nucleosome segregation and the inheritance of cellular identity**. *Nat Rev Genet* 2021, **22**:379–392, https://doi.org/10.1038/s41576-020-00312-w.

5. Eaton ML, Galani K, Kang S, Bell SP, MacAlpine DM: **Conserved nucleosome positioning defines replication origins**. *Genes Dev* 2010, **24**:748–753, https://doi.org/10.1101/gad.1913210.

6. Li S, Wasserman MR, Yurieva O, Bai L, O'Donnell ME, Liu S: **Nucleosome-directed replication origin licensing independent of a consensus DNA sequence**. *Nat Commun* 2022, **13**: 4947, https://doi.org/10.1038/s41467-022-32657-7.

7. * Chacin E, Reusswig K-U, Furtmeier J, Bansal P, Karl LA, Pfander B, Straub T, Korber P, Kurat CF: **Establishment and function of chromatin organization at replication origins**. *Nature* 2023, **616**: 836–842, https://doi.org/10.1038/s41586-023-05926-8.

This study demonstrates that *S. cerevisiae* ORC1 interacts with chromatin remodellers to establish an NDR flanked by a nucleosome array at origins *in vitro* and *in vivo*. This function is required for replication *in vitro* and for S phase entry *in vivo*. This work highlights that even in budding yeast, the chromatin organisation at origins is important for replication initiation.

8. Li S, Wasserman MR, Yurieva O, Bai L, O'Donnell ME, Liu S: **Origin recognition complex harbors an intrinsic nucleosome remodeling activity**. *Proc Natl Acad Sci USA* 2022, **119**, e2211568119, https://doi.org/10.1073/pnas.2211568119.

9. Ganier O, Prorok P, Akerman I, Méchali M: **Metazoan DNA replication origins**. *Curr Opin Cell Biol* 2019, **58**:134–141, https://doi.org/10.1016/j.ceb.2019.03.003.

10. Tian M, Wang Z, Su Z, Shibata E, Shibata Y, Dutta A, Zang C: **Integrative analysis of DNA replication origins and ORC-/MCM-binding sites in human cells reveals a lack of overlap**. *Elife* 2024, **12**, RP89548, https://doi.org/10.7554/eLife.89548.

11. * Poulet-Benedetti J, Tonnerre-Doncarli C, Valton A-L, Laurent M, Gérard M, Barinova N, Parisis N, Massip F, Picard F, Prioleau M-N: **Dimeric G-quadruplex motifs-induced NFRs determine strong replication origins in vertebrates**. *Nat Commun* 2023, **14**:4843, https://doi.org/10.1038/s41467-023-40441-4.
This work shows that dimeric G4 elements on the same strand can promote replication initiation at the $\beta^A$-globin origin in DT40 cells. This is accompanied by the presence of an NDR flanked by a well-positioned nucleosome at the origin. The association between dimeric G4 and nucleosome organisation at origins is also detected genome-wide in chicken, mouse and human cells, suggesting it may be a common mechanism to promote initiation in metazoans.

12. Costa A, Diffley JFX: **The initiation of eukaryotic DNA replication**. *Annu Rev Biochem* 2022, **91**:107–131, https://doi.org/10.1146/annurev-biochem-072321-110228.

13. Long H, Zhang L, Lv M, Wen Z, Zhang W, Chen X, Zhang P, Li T, Chang L, Jin C, *et al.*: **H2A.Z facilitates licensing and activation of early replication origins**. *Nature* 2020, **577**:576–581, https://doi.org/10.1038/s41586-019-1877-9.

14. Koyanagi E, Kakimoto Y, Minamisawa T, Yoshifuji F, Natsume T, Higashitani A, Ogi T, Carr AM, Kanemaki MT, Daigaku Y: **Global landscape of replicative DNA polymerase usage in the human genome**. *Nat Commun* 2022, **13**:7221, https://doi.org/10.1038/s41467-022-34929-8.

15. Vermeulen M, Eberl HC, Matarese F, Marks H, Denissov S, Butter F, Lee KK, Olsen JV, Hyman AA, Stunnenberg HG, *et al.*: **Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers**. *Cell* 2010, **142**:967–980, https://doi.org/10.1016/j.cell.2010.08.020.

16. Kimura H, Cook PR: **Kinetics of core histones in living human cells: little exchange of H3 and H4 and some rapid exchange of H2B**. *J Cell Biol* 2001, **153**.

17. * Flury V, Reverón-Gómez N, Alcaraz N, Stewart-Morgan KR, Wenger A, Klose RJ, Groth A: **Recycling of modified H2A-H2B provides short-term memory of chromatin states**. *Cell* 2023, S0092867423000077, https://doi.org/10.1016/j.cell.2023.01.007.
This study shows that in mouse ESCs the replisome component Pol$\alpha$ can recycle H2A-H2B dimers on the lagging strand independently of H3–H4. Restoration of modified (K119Ub) parental H2A-H2B supports the accurate reestablishment of H3K27me3. This puts forward the idea that H2A-H2B recycling can act as a short-term memory that helps restore longer-lived H3–H4 marks.

18. Clément C, Orsi GA, Gatto A, Boyarchuk E, Forest A, Hajj B, Miné-Hattab J, Garnier M, Gurard-Levin ZA, Quivy J-P, *et al.*: **High-resolution visualization of H3 variants during replication reveals their controlled recycling**. *Nat Commun* 2018, **9**:3181, https://doi.org/10.1038/s41467-018-05697-1.

19. Delaney K, Weiss N, Almouzni G: **The cell-cycle choreography of H3 variants shapes the genome**. *Mol Cell* 2023, **83**:3773–3786, https://doi.org/10.1016/j.molcel.2023.08.030.

20. * Gatto A, Forest A, Quivy J-P, Almouzni G: **HIRA-dependent boundaries between H3 variants shape early replication in mammals**. *Mol Cell* 2022, S1097276522002556, https://doi.org/10.1016/j.molcel.2022.03.017.
The authors show how in human cells the dual deposition of new replicative H3.1 and non-replicative H3.3 variants during S phase restores the pre-replicative landscape of the variants. It also establishes H3.3/H3.1 boundaries which correspond to early initiation zones. Loss of the H3.3-specific chaperone HIRA disrupts both the H3.3/H3.1 boundaries and early replication there, demonstrating the importance of the H3 variant balance for replication initiation.

21. Ray-Gallet D, Woolfe A, Vassias I, Pellentz C, Lacoste N, Puri A, Schultz DC, Pchelintsev NA, Adams PD, Jansen LET, *et al.*: **Dynamics of histone H3 deposition in vivo reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity**. *Mol Cell* 2011, **44**:928–941, https://doi.org/10.1016/j.molcel.2011.12.006.

22. Jin C, Felsenfeld G: **Nucleosome stability mediated by histone variants H3.3 and H2A**. *Z. Genes Dev.* 2007, **21**:1519–1529, https://doi.org/10.1101/gad.1547707.

23. Burton A, Torres-Padilla M-E: **Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis**. *Nat Rev Mol Cell Biol* 2014, **15**:723–735, https://doi.org/10.1038/nrm3885.

24. Theis A, Harrison MM: **Reprogramming of three-dimensional chromatin organization in the early embryo**. *Curr Opin Struct Biol* 2023, **81**, 102613, https://doi.org/10.1016/j.sbi.2023.102613.

25. * Nakatani T, Schauer T, Altamirano-Pacheco L, Klein KN, Ettinger A, Pal M, Gilbert DM, Torres-Padilla M-E: **Emergence of replication timing during early mammalian development**. *Nature* 2023, https://doi.org/10.1038/s41586-023-06872-1.
Using single-cell Repli-seq, the authors investigate replication timing in the early mammalian embryo for the first time. This reveals that the RT programme is not well-defined in mouse zygotes and 2-cell stage embryos, but becomes gradually less variable as development progresses. As transcriptional programmes, chromatin state and genome organisation are not established in the early embryo, this model provides an opportunity to disentangle their regulation.

26. Halliwell JA, Martin-Gonzalez J, Hashim A, Dahl JA, Hoffmann ER, Lerdrup M: **Sex-specific DNA-replication in the early mammalian embryo**. *Preprint at bioRxiv* 2024, https://doi.org/10.1101/2023.12.22.572993. 10.1101/2023.12.22.572993.

27. Xu S, Wang N, Zuccaro MV, Gerhardt J, Baslan T, Koren A, Egli D: **DNA replication in early mammalian embryos is patterned, predisposing lamina-associated regions to fragility**. *Preprint at bioRxiv* 2023, https://doi.org/10.1101/2023.12.25.573304. 10.1101/2023.12.25.573304.

28. Ishiuchi T, Abe S, Inoue K, Yeung WKA, Miki Y, Ogura A, Sasaki H: **Reprogramming of the histone H3.3 landscape in the early mouse embryo**. *Nat Struct Mol Biol* 2020, https://doi.org/10.1038/s41594-020-00521-1.

29. Nakatani T, Lin J, Ji F, Ettinger A, Pontabry J, Tokoro M, Altamirano-Pacheco L, Fiorentino J, Mahammadov E, Hatano Y, *et al.*: **DNA replication fork speed underlies cell fate changes and promotes reprogramming**. *Nat Genet* 2022, https://doi.org/10.1038/s41588-022-01023-0.

30. Goekbuget D, Boileau RM, Lenshoek K, Blelloch R: **MLL3/MLL4 enzymatic activity shapes DNA replication timing**. *Preprint at bioRxiv* 2023, https://doi.org/10.1101/2023.12.07.569680. 10.1101/2023.12.07.569680.

31. Stewart-Morgan KR, Petryk N, Groth A: **Chromatin replication and epigenetic cell memory**. *Nat Cell Biol* 2020, **22**:361–371, https://doi.org/10.1038/s41556-020-0487-y.

32. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, Nakatani Y, Dent SYR, Almouzni G: **The HP1$\alpha$–CAF1–SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin**. *EMBO Rep* 2009, **10**:769–775, https://doi.org/10.1038/embor.2009.90.

33. Cheng L, Zhang X, Wang Y, Gan H, Xu X, Lv X, Hua X, Que J, Ordog T, Zhang Z: **Chromatin Assembly Factor 1 (CAF-1) facilitates the establishment of facultative heterochromatin during pluripotency exit**. *Nucleic Acids Res* 2019, gkz858, https://doi.org/10.1093/nar/gkz858.

34. * Chen B, MacAlpine HK, Hartemink AJ, MacAlpine DM: **Spatio-temporal kinetics of CAF-1-dependent chromatin maturation ensures transcription fidelity during S-phase**. *Genome Res* 2023, https://doi.org/10.1101/gr.278273.123.

The authors show a direct role for nucleosome assembly by CAF-1 for transcriptional repression in S phase in *S. cerevisiae*. This work highlights that nucleosome density can promote repression independently of heterochromatin-associated histone modifications.

35. Dreyer J, Ricci G, Van Den Berg J, Bhardwaj V, Funk J, Armstrong C, Van Batenburg V, Sine C, Vaninsberghe MA, Marsman R, *et al.*: **Acute multi-level response to defective de novo chromatin assembly in S-phase**. *Preprint* 2024, https://doi.org/10.1101/2024.03.22.586291. 10.1101/2024.03.22.586291.

36. Carraro M, Hendriks IA, Hammond CM, Solis-Mezarino V, Völker-Albert M, Elsborg JD, Weisser MB, Spanos C, Montoya G, Rappsilber J, *et al.*: **DAXX adds a de novo H3.3K9me3 deposition pathway to the histone chaperone network**. *Mol Cell* 2023, S1097276523001077, https://doi.org/10.1016/j.molcel.2023.02.009.

37. Bellelli R, Belan O, Pye VE, Clement C, Maslen SL, Skehel JM, Cherepanov P, Almouzni G, Boulton SJ: **POLE3-POLE4 is a histone H3-H4 chaperone that maintains chromatin integrity during DNA replication**. *Mol Cell* 2018, **72**:112–126.e5, https://doi.org/10.1016/j.molcel.2018.08.043.

38. Yu C, Gan H, Serra-Cardona A, Zhang L, Gan S, Sharma S, Johansson E, Chabes A, Xu R-M, Zhang Z: **A mechanism for preventing asymmetric histone segregation onto replicating DNA strands**. *Science* 2018, **361**:1386–1389, https://doi.org/10.1126/science.aat8849.

39. Huang H, Strømme CB, Saredi G, Hödl M, Strandsby A, González-Aguilera C, Chen S, Groth A, Patel DJ: **A unique binding mode enables MCM2 to chaperone histones H3–H4 at replication forks**. *Nat Struct Mol Biol* 2015, **22**:618–626, https://doi.org/10.1038/nsmb.3055.

40. Petryk N, Dalby M, Wenger A, Stromme CB, Strandsby A, Andersson R, Groth A: **MCM2 promotes symmetric inheritance of modified histones during DNA replication**. *Science* 2018, **361**:1389–1392, https://doi.org/10.1126/science.aau0294.

41. Groth A, Corpet A, Cook AJL, Roche D, Bartek J, Lukas J, Almouzni G: **Regulation of replication fork progression through histone supply and demand**. *Science* 2007, **318**:1928–1931, https://doi.org/10.1126/science.1148992.

42. Gan H, Serra-Cardona A, Hua X, Zhou H, Labib K, Yu C, Zhang Z: **The mcm2-ctf4-polα Axis facilitates parental histone H3-H4 transfer to lagging strands**. *Mol Cell* 2018, **72**:140–151.e3, https://doi.org/10.1016/j.molcel.2018.09.001.

43. Li Z, Hua X, Serra-Cardona A, Xu X, Gan S, Zhou H, Yang W-S, Chen C, Xu R-M, Zhang Z: **DNA polymerase alpha interacts with H3-H4 and facilitates the transfer of parental histones to lagging strands**. *Sci Adv* 2020, **6**:1–14, https://doi.org/10.1126/sciadv.abb5820.

44. Serra-Cardona A, Hua X, McNutt SW, Zhou H, Toda T, Jia S, Chu F, Zhang Z: **The PCNA–Pol δ complex couples lagging strand DNA synthesis to parental histone transfer for epigenetic inheritance**. *Sci Adv* 2024, **10**, eadn5175, https://doi.org/10.1126/sciadv.adn5175.

45. Tian C, Zhang Q, Jia J, Zhou J, Zhang Z, Karri S, Jiang J, Dickinson Q, Yao Y, Tang X, *et al.*: **DNA polymerase delta governs parental histone transfer to DNA replication lagging strand**. *Proc Natl Acad Sci U S A* 2024, **121**, e2400610121, https://doi.org/10.1073/pnas.2400610121.

46. Flury V, Groth A: **Safeguarding the epigenome through the cell cycle: a multitasking game**. *Curr Opin Genet Dev* 2024, **85**, 102161, https://doi.org/10.1016/j.gde.2024.102161.

47. Fang Y, Hua X, Shan C-M, Toda T, Qiao F, Zhang Z, Jia S: **Coordination of histone chaperones for parental histone segregation and epigenetic inheritance**. *Genes Dev* 2024, https://doi.org/10.1101/gad.351278.123. genesdev;gad.351278.123v1.

48. Escobar TM, Yu J-R, Liu S, Lucero K, Vasilyev N, Nudler E, Reinberg D: ***Inheritance of repressed chromatin domains during S-phase requires the histone chaperone NPM1 (molecular biology)***. https://doi.org/10.1101/2021.08.31.458436.

49. Li Z, Duan S, Hua X, Xu X, Li Y, Menolfi D, Zhou H, Lu C, Zha S, Goff SP, *et al.*: **Asymmetric distribution of parental H3K9me3 in S phase silences L1 elements**. *Nature* 2023, **623**:643–651, https://doi.org/10.1038/s41586-023-06711-3.

50. * Wen Q, Zhou J, Tian C, Li X, Song G, Gao Y, Sun Y, Ma C, Yao S, Liang X, *et al.*: **Symmetric inheritance of parental histones contributes to safeguarding the fate of mouse embryonic stem cells during differentiation**. *Nat Genet* 2023:1–12, https://doi.org/10.1038/s41588-023-01477-w.
This study, along with Wenger et al. (2023) investigated the functional importance of symmetric histone recycling in mouse ESCs. Biased recycling showed a redistribution of H3K27me3 at developmental genes and impaired differentiation capacity, demonstrating the significance of symmetric inheritance for cell state.

51. Torné J, Ray-Gallet D, Boyarchuk E, Garnier M, Le Baccon P, Coulon A, Orsi GA, Almouzni G: **Two HIRA-dependent pathways mediate H3.3 de novo deposition and recycling during transcription**. *Nat Struct Mol Biol* 2020, **27**:1057–1068, https://doi.org/10.1038/s41594-020-0492-7.

52. Ray-Gallet D, Ricketts MD, Sato Y, Gupta K, Boyarchuk E, Senda T, Marmorstein R, Almouzni G: **Functional activity of the H3.3 histone chaperone complex HIRA requires trimerization of the HIRA subunit**. *Nat Commun* 2018, **9**:3103, https://doi.org/10.1038/s41467-018-05581-y.

53. * Mendiratta S, Ray-Gallet D, Lemaire S, Gatto A, Forest A, Kerlin MA, Almouzni G: **Regulation of replicative histone RNA metabolism by the histone chaperone ASF1**. *Mol Cell* 2024, https://doi.org/10.1016/j.molcel.2023.12.038.
This work explores how the supply of new histones and nucleosome assembly are coordinated in S phase by histone chaperones. This reveals that the general H3–H4 chaperone ASF1 which handles both new and parental histones also regulates replicative histone mRNA processing. This suggests ASF1 can act as a 'chaperone checkpoint' to fine-tune histone levels which may contribute to balance deposition of new histones with recycling.

54. * Wenger A, Biran A, Alcaraz N, Redó-Riveiro A, Sell AC, Krautz R, Flury V, Reverón-Gómez N, Solis-Mezarino V, Völker-Albert M, *et al.*: **Symmetric inheritance of parental histones governs epigenome maintenance and embryonic stem cell identity**. *Nat Genet* 2023:1–12, https://doi.org/10.1038/s41588-023-01476-x.
This study, along with Wen et al. (2023) investigated the biological significance of disrupting symmetric parental histone transfer in mouse ESCs. This led to reorganisation of H3K9me3 and H3K27me3, linked to misexpression of transposable elements and developmental genes, respectively. It also impaired the capacity of the cells to explore different state and to exit pluripotency normally, indicating the importance of symmetric recycling for normal cell function.

55. Attali I, Botchan MR, Berger JM: **Structural mechanisms for replicating DNA in eukaryotes**. *Annu Rev Biochem* 2021, **90**:77–106, https://doi.org/10.1146/annurev-biochem-090120-125407.

56. Moggs JG, Grandi P, Quivy J-P, Jónsson ZO, Hübscher U, Becker PB, Almouzni G: **A CAF-1–PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage**. *Mol Cell Biol* 2000, **20**:1206–1218, https://doi.org/10.1128/MCB.20.4.1206-1218.2000.

57. Rouillon C, Eckhardt BV, Kollenstart L, Gruss F, Verkennis AEE, Rondeel I, Krijger PHL, Ricci G, Biran A, van Laar T, *et al.*: **CAF-1 deposits newly synthesized histones during DNA replication using distinct mechanisms on the leading and lagging strands**. *Nucleic Acids Res* 2023, **51**:3770–3792, https://doi.org/10.1093/nar/gkad171.

58. Xu X, Hua X, Brown K, Ren X, Zhang Z: **Mcm2 promotes stem cell differentiation via its ability to bind H3-H4**. *Elife* 2022, **11**, e80917, https://doi.org/10.7554/eLife.80917.

59. Tian C, Zhou J, Li X, Gao Y, Wen Q, Kang X, Wang N, Yao Y, Jiang J, Song G, *et al.*: **Impaired histone inheritance promotes tumor progression**. *Nat Commun* 2023, **14**:3429, https://doi.org/10.1038/s41467-023-39185-y.

60. Sarthy JF, Meers MP, Janssens DH, Henikoff JG, Feldman H, Paddison PJ, Lockwood CM, Vitanza NA, Olson JM, Ahmad K, *et al.*: **Histone deposition pathways determine the chromatin landscapes of H3.1 and H3.3 K27M oncohistones**. *Elife* 2020, **9**, e61090, https://doi.org/10.7554/eLife.61090.

61. Ahmad K, Henikoff S: **The H3.3K27M oncohistone antagonizes reprogramming in Drosophila**. *PLoS Genet* 2021, **17**, e1009225, https://doi.org/10.1371/journal.pgen.1009225.

62. Zasadzińska E, Huang J, Bailey AO, Guo LY, Lee NS, Srivastava S, Wong KA, French BT, Black BE, Foltz DR: **Inheritance of CENP-A nucleosomes during DNA replication requires HJURP**. *Dev Cell* 2018, **47**:348–362.e7, https://doi.org/10.1016/j.devcel.2018.09.003.

63. Foltman M, Evrin C, De Piccoli G, Jones RC, Edmondson RD, Katou Y, Nakato R, Shirahige K, Labib K: **Eukaryotic replisome components cooperate to process histones during chromosome replication**. *Cell Rep* 2013, **3**:892–904, https://doi.org/10.1016/j.celrep.2013.02.028.

64. Safaric B, Chacin E, Scherr MJ, Rajappa L, Gebhardt C, Kurat CF, Cordes T, Duderstadt KE: **The fork protection complex recruits FACT to reorganize nucleosomes during replication**. *Nucleic Acids Res* 2022, **50**:1317–1334, https://doi.org/10.1093/nar/gkac005.

65. Wang X, Tang Y, Xu J, Leng H, Shi G, Hu Z, Wu J, Xiu Y, Feng J, Li Q: **The N-terminus of Spt16 anchors FACT to MCM2–7 for parental histone recycling**. *Nucleic Acids Res* 2023, **51**: 11549–11567, https://doi.org/10.1093/nar/gkad846.

66. Liu S, Xu Z, Leng H, Zheng P, Yang J, Chen K, Feng J, Li Q: **RPA binds histone H3-H4 and functions in DNA replication–coupled nucleosome assembly**. *Science* 2017, **355**:415–420, https://doi.org/10.1126/science.aah4712.

67. Smith S, Stillman B: **Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication in vitro**. *Cell* 1989, **58**:15–25, https://doi.org/10.1016/0092-8674(89)90398-X.

68. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y: **Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis**. *Cell* 2004, **116**:51–61, https://doi.org/10.1016/S0092-8674(03)01064-X.

69. Banaszynski LA, Wen D, Dewell S, Whitcomb SJ, Lin M, Diaz N, Elsässer SJ, Chapgier A, Goldberg AD, Canaani E, *et al.*: **Hira-dependent histone H3.3 deposition facilitates PRC2 recruitment at developmental loci in ES cells**. *Cell* 2013, **155**: 107–120, https://doi.org/10.1016/j.cell.2013.08.061.

70. Pchelintsev NA, McBryan T, Rai TS, van Tuyn J, Ray-Gallet D, Almouzni G, Adams PD: **Placing the HIRA histone chaperone complex in the chromatin landscape**. *Cell Rep* 2013, **3**: 1012–1019, https://doi.org/10.1016/j.celrep.2013.03.026.

71. Goldberg AD, Banaszynski LA, Noh K-M, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X, *et al.*: **Distinct factors control histone variant H3.3 localization at specific genomic regions**. *Cell* 2010, **140**:678–691, https://doi.org/10.1016/j.cell.2010.01.003.

72. Müller S, Almouzni G: **Chromatin dynamics during the cell cycle at centromeres**. *Nat Rev Genet* 2017, **18**:192–208, https://doi.org/10.1038/nrg.2016.157.