The Histone H3 Family and Its Deposition Pathways

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Chapter 2: The histone H3 family and its deposition pathways

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Abstract

Within the cell nucleus, the organization of the eukaryotic DNA into chromatin uses histones as components of its building block, the nucleosome. This chromatin organization contributes to the regulation of all DNA template-based reactions impacting genome function, stability and plasticity. Histones and their variants endow chromatin with unique properties and show a distinct distribution into the genome that is regulated by dedicated deposition machineries. The histone variants have important roles during early development, cell differentiation and chromosome segregation. Recent progress has also shed light on how mutations and transcriptional deregulation of these variants participate in tumorigenesis. In this chapter we introduce the organization of the genome in chromatin with a focus on the basic unit, the nucleosome, which contains histones as the major protein component. Then we review our current knowledge on the histone H3 family and its variants - in particular H3.3 and CenH3^{CENP-A}- focusing on their deposition pathways and their dedicated histone chaperones that are key players in histone dynamics.

Keywords

Chromatin, Nucleosome, Histone, Histone Variant, Histone Chaperone, Histone Deposition

Abbreviations

ChIP-seq	chromatin immunoprecipitation sequencing
DSC	DNA synthesis coupled
DSI	DNA synthesis independent
ES	embryonic stem
KO	knockout
NCP	nucleosome core particle
PTM	post-translational modification

2.1 Introduction

2.1.1 Chromatin

The term chromatin (from the Greek *chrôma*, “color”) emerged in the 1880s when Flemming found a structure in the cell nucleus that strongly absorbed basophilic dyes [1]. Chromatin is a complex nucleoprotein structure comprising mainly DNA (Deoxyribonucleic acid) and basic proteins (histones). DNA is the heritable genetic material (genome) which consists of about three billion base pairs distributed into 46 chromosomes per cell in human. This material representing about two meters of DNA is confined in each cell in a nuclear compartment of few micrometers in diameter. Thus, chromatin organization ensures to compact DNA from the basic unit, the nucleosome, up to higher level of architecture. Chambon and Kornberg discovered in the 1970s the nucleosome as a repeating unit for the organization of chromatin [2,3]. This nucleosome comprises about 147 bp (base pairs) of DNA wrapped around a core histone octamer flanked by 20-90 bp of a linker DNA associated with the linker histone H1. The complex 147 bp DNA-core

histone octamer (without the linker DNA and H1) constitutes the nucleosome core particle (NCP). The core histone octamer consists of two copies of each core histone H3, H4, H2A and H2B organized into a $(H3\text{-}H4)_2$ tetramer flanked by two (H2A-H2B) dimers. Resolution of the crystal structure of the nucleosome core particle at 2.8 Å in 1997 revealed how histones interact with each other and with DNA, and how their amino-terminal tails protrude out of the particle [4]. The nucleosome array forms a 10 nm (nanometer) diameter fiber that resembles "beads on a string" [5]. In the cell, this fiber undergoes different levels of compaction to form the higher order chromatin structure. In 1928, Heitz observed in the cell nucleus two different types of regions with a light microscope, discrete highly condensed regions and dispersed lightly packed regions that constitute two types of chromatin, heterochomatin and euchromatin, respectively [6]. Constitutive heterochromatin mainly consists of repetitive DNA sequences that do not contain genes such as telomeres, centromeres and pericentromeres, whereas euchromatin is mainly comprised of the coding part of the genome harboring genes. Recent progress with chromatin capture technologies has further revealed levels of chromatin organization with interacting chromatin loops and topologically associating domains (TADs) which serve as functional platforms for physical interactions between regulatory elements [7,8].

Beyond DNA compaction, chromatin organization influences all nuclear functions. Indeed, chromatin is the substrate for the different processes operating on DNA such as replication, transcription and repair. Thus a proper control of the dynamics of this organization ensures accurate genome function [9-11]. This control is exerted at all levels from the DNA and histones within the nucleosome particle up to the higher order chromatin architecture in the cell nucleus [12].

2.1.2 Histones

Histones are small basic proteins that are among the most conserved in eukaryotes [13]. The core histones from the H2A, H2B, H3 and H4 families range in size from 11 kDa to 15 kDa while the linker histones from the H1 family are around 21 kDa. A structurally conserved motif called histone-fold domain is present in all core histones. It consists of three α-helices (α1, α2 and α3) connected by short loops L1 and L2 that mediate heterodimeric interactions between the core histones [14]. The unstructured N-terminal extremity that extends at the surface of the nucleosome is the main region of the histone which is subjected to post-translational modifications (PTMs) with important consequences on chromatin functions [15]. Numerous PTMs include acetylation, phosphorylation, methylation, ubiquitylation, crotonylation and the latest described serotonylation [16]. As schematic examples, tri-methylation of the lysine 9 of histone H3 (H3K9me3) mainly associates with silenced regions in the genome while tri-methylation of the lysine 4 of histone H3 (H3K4me3) generally correlates with transcriptionally active regions. These histone PTMs do generally occur in various combinations that gave rise to the hypothesis of a histone code where these modifications would work sequentially and/or together [17,18]. They can either modulate the physical properties of the nucleosome and/or regulate the binding of protein partners that recognize specific modifications or RNA and possibly alter higher order structure. The histone PTMs are most often reversible providing a system to react to external stimuli for short term response, as part of a signaling module. They can also be maintained over cellular divisions and thereby function as a memory module of "epigenetic" nature. Furthermore, as we will discuss, in addition to these PTMs, the choice of distinct histone variants to form a nucleosome particle offers not only various provision of histones but also another way to alter the nature of the NCP with impact on chromatin function.

2.1.3 Replicative Histones and Histone Variants

In most eukaryotes, two types of histones exist for each histone family, the replicative and the non-replicative histones, the latter commonly referred to as non-allelic histone variants [19]. The replicative histones exhibit a high peak of expression during S phase when the doubling of genomic content requires a massive provision of histones. The genes of replicative histones show a peculiar organization in clusters that comprise multiple copies of all core histones and the H1 histone linker [20,21]. This unique genomic organization and regulation at multiple levels contribute to optimize coregulation which is essential for the need of a high peak of expression of replicative histones during S phase. These histone clusters lack introns, have relatively short UTRs, and produce transcripts that do not undergo polyadenylation and harbor a conserved 3' stem-loop structure which is required for the regulation of mRNA stability [22]. The existence of non-allelic variants for H2A, H2B and H3 in mammals was first uncovered in 1977 when resolving histones on polyacrylamide gel electrophoresis in presence of non-ionic detergents (Triton Acid Urea gels) [23]. In contrast with replicative histones, the expression of histone variants does not increase during S phase and each variant harbours a unique temporal expression. They are encoded by multi-exon genes located outside of histone clusters. Transcripts lack 3' stem-loop and undergo conventional processing through splicing and polyadenylation like most other RNA polymerase II (Pol II) transcripts. The protein sequence of a histone variant is either extremely similar or divergent from its replicative counterparts. Importantly, not only the expression but also the deposition pathways for the replicative histones and the histone variants are distinct. The replicative histones are incorporated into chromatin in a DNA synthesis coupled (DSC) manner. This occurs mainly at replication forks during S phase when vast amounts of newly synthesized histones are required to ensure chromatin restoration on the duplicated genome. In contrast, the histone variants are incorporated into chromatin in a DNA synthesis independent (DSI) manner. Their mode of deposition onto DNA and their location in the genome are in general specific for each histone variant [24].

The deregulated expression of histone variants and histone chaperones in various cancers shed light on the importance to connect chromatin and genome stability [25,26]. The discovery of H3 mutations in pediatric glioblastomas attracted attention on histone variants in cancer development [27,28]. In particular, mutations of histone H3 at K27M and G34V/R known as “oncohistones” were put forward as major drivers in these glioblastomas [29-32]. Since then, a considerable number of histone mutations - higher than previously recognized- has been identified in human tumours [33]. An increased interest has thus arisen in exploring the histones of the H3 family in greater details along with their modes of incorporation into chromatin by their dedicated chaperones. Progress in this field is therefore of critical importance for understanding normal development and disease like cancer [25].

2.2 The Histone H3 Family

To date in human, eight members constitute the histone H3 family, two replicative histones (H3.1 and H3.2) and six histone variants. Among these H3 variants, limited information exists for the testis-specific H3.4 (H3.1t) [34], the hominid-specific H3.5 [35] and the two primate-specific H3.X and H3.Y [36]. Thus, we will focus on the H3.1 and H3.2 replicative histones and H3.3 and CenH3^{CENP-A} histone variants which have been explored in more details.

2.2.1 Replicative H3.1 and H3.2

The two replicative histones H3, H3.1 and H3.2, are encoded by several genes in clusters **(Fig. 2.1).** They differ by only one residue at position 96 **(Fig. 2.2A).** Most studies have focused on H3.1 as the representative of H3 replicative histone in mammals, yet H3.2 is the most common replicative histone in eukaryotes. H3.1 is present only in mammals in addition to H3.2. Despite their high similarity, in human, some functional specificity was suggested based on differences in both expression patterns and associated PTMs [37]. As expected for replicative histones, both H3.1 and H3.2 are incorporated into chromatin at replication forks during S phase in a DSC manner in order to duplicate chromatin of the replicated DNA [38-40]. Moreover, deposition of

H3.1 outside of S phase occurs at sites of DNA synthesis as observed at UV damaged sites [41]. Indeed, the DNA repair process leads to histones eviction to allow access to the repair machinery and repaired coupled incorporation of H3.1 participates to the chromatin restoration [42]. This is accompanied by a recycling of pre-existing histones (Adam et al, 2017).

2.2.2 H3.3 Variant

The histone variant H3.3 is encoded by two single genes in mammals **(Fig. 2.1)**. It is closely related to H3.1 and H3.2 with only five and four amino acid residue differences, respectively **(Fig. 2.2 A)**. One difference concerns the residue 31 in the N-terminal tail of the histones with a serine in H3.3 instead of an alanine in both H3.1 and H3.2. This serine 31 in H3.3 is phosphorylated during mitosis [43] and at transcribing regions in mouse activated macrophages [44], however the exact role of this modification remains elusive. The three other different residues AIG, located in the α2 helix of the histone fold (at positions 87, 89 and 90), are important for specific histone chaperone recognition and the choice of a deposition pathway. An evolutionary analysis suggested that H3.3 is the ancestral form of the replicative H3.1/2 and in budding yeast the unique non-centromeric histone H3 is closely related to H3.3 [19]. However, recent work to "humanize" histones in budding yeast showed that adaptation to H3.1 proved easier in yeast compared to H3.3 in the context of a fully humanized nucleosome [45]. In mammals, two paralogous genes, H3.3A and H3.3B, encode the same H3.3 protein but have different codons (could impact the folding) and distinct untranslated regions (could impact transcription regulation) **(Fig. 2.1)**. This suggests that a distinct transcriptional and post-transcriptional regulation of these two genes could provide different patterns of expression among tissues and during development [46-48]. The H3.3 variant present throughout the cell cycle was first described for its high level of incorporation at active rDNA arrays independently of replication in *Drosophila* [38]. H3.3 is deposited onto DNA in a DSI manner during interphase (G1, S and G2 phases). H3.3 is the histone H3 predominantly present in chromatin of cells that are not dividing like quiescent or post-mitotic cells, due to its capacity to be incorporated in a pathway

independent of DNA synthesis [49-51]. Moreover, upon fertilization and concomitantly with protamines removal, a major reprogramming involves H3.3 incorporation in paternal chromatin before the first round of DNA replication in both *Drosophila* and mouse [52-54]. The genome wide distribution of H3.3 variant as observed by ChIP-seq analysis in mammalian cells shows a distinct pattern. Enriched in euchromatin at active genes, H3.3 presence is most often correlated with active transcription [55]. More precisely, H3.3 accumulates throughout the body of active genes but also at promoter regions at both active and inactive genes and at genic and intergenic regulatory regions in ES cells. In addition, a significant contribution of this variant is also revealed through enrichment in heterochromatin at both telomere and pericentric heterochromatin in ES cells [55,56]. Remarkably, the crystal structure of human nucleosome core particles containing H3.1, H3.2 or H3.3 revealed identical structures suggesting a common function in the organization at the level of individual particles **(Fig. 2.2 B)** [57]. However, H3.3 containing nucleosomes *in vivo* appear more sensitive to salt-dependent disruption [58], arguing that the presence of this variant, the associated PTMs or combination with other variants (such as H2AZ) may change the properties of the nucleosome towards a more open/active chromatin [59]. Importantly, morpholino experiments revealed a critical role for H3.3 during early development in *X. laevis* [60]. In mice, a double-KO of the two genes (H3.3A and H3.3B) results in impaired development and embryonic lethality, no double-KO embryo surviving after stage E8.5 [47]. These defects are proposed to result from heterochromatin structures dysfunction at telomeres and centromeres leading to mitotic defects [47]. How this is entailed remains to be deciphered since it is unclear to which extent these phenotypes arise from the provision of the variant, the deposition mode or the final organization involving the variant or a combination.

2.2.3 CenH3^{CENP-A} Variant

In 1985, Earnshaw and Rothfield identified CenH3^{CENP-A} as one of the proteins detected by autoantibodies from patients with CREST (calcinosis, Reynaud syndrome, oesophageal dysmotility, sclerodactyly, telangiectasia) [61]. Then, in 1991 Palmer et al. demonstrated that

CenH3^{CENP-A} is a distinctive histone, with some sequences similarity to H3 [62]. This distant histone H3 variant was also called “deviant” given the fact that many segments are not related to H3 [63]. The histone variant CenH3^{CENP-A} is encoded by a single gene **(Fig. 2.1)** and exhibits less than 50% of amino acid sequence identity with its replicative counterparts H3.1/2 and is highly divergent in various species **(Fig. 2.2 A)**. This low level of conservation is in line with a rapid evolution of centromere organization and its components [64]. While H3.1 and H3.3 nucleosomal structures are almost identical, the more compact CenH3^{CENP-A} nucleosome only wraps 121 bp of DNA, and this may impact further the higher-order chromatin organization in these regions **(Fig. 2.2 B)** [65-67]. In human, expression of new CenH3^{CENP-A} occurs in G2/M phases. Its incorporation into chromatin is restricted to late mitosis (telophase)/early G1 by a DSI pathway, leading to centromeric CenH3^{CENP-A} dilution during replication [68]. The deposition of CenH3^{CENP-A} specifically at centromere (in the centric region) plays a crucial role in chromosome segregation by enabling kinetochore formation in mitosis [69,70]. This critical need is illustrated in KO mice which are not able to develop beyond the stage E8.5 [71]. The embryos accumulate mitotic problems, further arguing for a major function of this variant in chromosome segregation.

2.3 Histone H3-H4 Chaperones

2.3.1 Histone Chaperone Definition

In the NCP, the basic charge of the histones is neutralized by the phosphate backbone of the DNA. Before incorporation into chromatin or after eviction, free histone in solution could potentially, due to their charge, engage into promiscuous interactions with any acidic partner and even could form aggregates in the cell. This is prevented by dedicated proteins, named histone chaperones which escort non-nucleosomal histones in the cell throughout all their cellular life [72]. Some of them directly buffer the positive charge. Nucleoplasmin, the most prominent protein in *X.laevis* oocyte, thanks to its properties to promote chromatin assembly, was the first protein named “histone chaperone” in 1977 by Laskey [73]. The current definition of a histone

chaperone is “a protein that associates with histones and is involved in their transfer but is not necessarily part of the final product” [74]. This definition fully illustrates the general property of a histone chaperone, and underlines the fact that *in vivo*, histones are never left alone from their synthesis to their delivery into or eviction from chromatin. All processes involving histone transfer or modification will thus involve at least one histone chaperone. They have a wide range of functions including histone transport, buffering, storage, histone modification, recycling and deposition onto DNA as well as nucleosome remodeling. *In vitro*, all histone chaperones share the fundamental ability to promote a progressive transfer of purified histones onto naked DNA at physiological ionic strength to reconstitute nucleosomes from purified components [75]. Interestingly, no single feature in term of sequence allows to demarcate a protein as a histone chaperone and some proteins turned out to function as histone chaperones after having been first characterized for other functions. Among histone chaperones, we can consider a first category according to affinity for either H2A-H2B or H3-H4. Then, within these categories, a further distinction depends on the selectivity for replicative histones and/or for one or several particular histone variants. Here, we focus on H3-H4 chaperones with an emphasis on those involved in histone deposition using newly synthesized histones (new/*de novo* deposition) or old histones (recycling) **(Table 2.1)** (for reviews on histone chaperones [24,76,77]).

2.3.2 Dedicated H3-H4 Chaperones

2.3.2.1 H3.1/2-H4 Chaperone

The chromatin assembly factor 1 (CAF-1) is the unique histone chaperone complex that interacts selectively with the replicative variants H3.1/2 **(Fig. 2.3)**. The CAF-1 complex was identified in 1989 on the basis of its ability to promote specifically nucleosome assembly *in vitro* onto newly synthesized DNA during replication with cytosolic extracts derived from human cells [78]. It consists of three distinct subunits p150/CHAF1A , p60/CHAF1B and p48/RbAp48/RBBP4 also referred to as “large”, “mid” and “small” subunits. They are functionally conserved in *S.cerevisiae* as CAC1, CAC2 and CAC3, respectively. The “large” subunit, p150, provides a

scaffold for the other CAF-1 subunits and mediates recruitment of the complex and interaction with other nuclear factors. In particular, its N-terminal portion contains two important regions: a stretch that is enriched in K/E/R amino acids (KER) predicted to bind DNA, and a proliferating cell nuclear antigen (PCNA) interacting peptide (PIP-box) motif [79,80]. An oscillation between monomeric and homo-oligomeric forms of p150 participates in the regulation of the functional activity of CAF-1 [81,82]. In addition to histone binding, p150 interacts with heterochromatin protein 1 (HP1), an interaction of importance for the replication of pericentric heterochromatin [83,84].The “mid” subunit, p60, with a WD40 propeller fold involved in the binding of H3-H4 dimer, is responsible for histone loading. The “small” subunit, p48, provides less-well characterized accessory interactions and is part of several other chromatin regulating complexes such as the corepressor mSin3A [85]. It can possibly serve as an interface or link between various complexes. The H3.1 complex purified from human cell extracts retrieved all three CAF-1 subunits required for the deposition of H3.1 onto DNA coupled with DNA synthesis [86] as found later with the H3.2 complex purification [40]. Thus, the deposition of both H3 replicative histones relies on CAF-1. The current model for histone deposition promoted by CAF-1 involves that the complex binds an H3-H4 dimer and that a transient association of two CAF-1-H3-H4 allows two histone chaperone complexes to concertedly deposit one $(H3\text{-}H4)_2$ tetramer onto DNA [87]. Loss of p150 CAF-1 in homozygous mutants leads to very early developmental arrest at the 16-cell stage in mice (between stages E2.0 and E3.0). These embryos show severe alterations in the organization of cell nuclei and their constitutive heterochromatin [88]. In ES cells, downregulation of CAF-1 can favor the emergence of cells showing properties of totipotent cells [89]. In somatic cells, induction to pluripotency IPS cells is facilitated when CAF-1 is reduced [90]. In T cells, CAF-1 cooperate with DNA methyltransferases and histone modifying enzymes to maintain silent states of the Cd4 gene [91]. This is in line with the general view according to which CAF-1 can contribute to the maintenance of somatic cell identity by stabilizing chromatin patterns [11].

2.3.2.2 H3.3-H4 Chaperones

H3.3 variants present two selective H3.3 histone chaperones: the Histone regulator A (HIRA) complex and death domain-associated protein 6 - α-thalassaemia/mental retardation syndrome X-linked (DAXX-ATRX) (for review [92,93]) **(Fig. 2.3)**.

The HIRA gene was identified in 1995 within a region of chromosome 22q11.2 deleted in most patients with a developmental disorder, the DiGeorge syndrome [94]. The HIRA acronym comes from its amino acid sequence homology to the two *S. cerevisiae* proteins histone regulation 1 and 2 (Hir1p and Hir2p). Initially described as a chaperone involved in a DSI nucleosome assembly pathway using the *X. laevis* egg extract model system [95], the identification of HIRA in the purifed H3.3 complex revealed its dedicated function in the deposition of H3.3 [86]. In addition to HIRA, two other proteins, ubinuclein 1 (UBN1) and calcineurin-binding protein 1 (CABIN1), co-purified with H3.3 and turned out later to be part of the HIRA histone chaperone complex [96,97]. Both UBN1 and CABIN1 interact with HIRA which plays therefore a central platform role in the complex. UBN1, first identified as a nuclear protein interacting with cellular and viral transcription factors [98], is the subunit that directly interacts with the H3.3-H4 dimer [99]. X-ray crystallographic analysis, revealed that the Hpc2-related domain (HRD) in UBN1 binds H3.3 in the proximity of the three residues AIG at positions 87-89-90 (in the α2 helix of the histone fold domain) that are different between H3.1/2 and H3.3 [100]. The Gly90 in H3.3 mediates the specificity for binding to H3.3-H4 over H3.1-H4. CABIN1 was first described as a corepressor of the MEF2 family of transcription factors [101]. To date, its exact function within the HIRA complex remains unclear. Like HIRA, UBN1 and CABIN1 have *S. cerevisiae* counterparts, histone periodic control 2 (Hpc2p) and histone regulation 3 (Hir3p), respectively. They form with Hir1p and Hir2p, the Hir complex which is involved in the incorporation of H3 independently of DNA synthesis in yeast [102]. The HIRA subunit forms a homotrimer that interacts with two CABIN1 subunits [103]. This trimeric structure is required for the functional activity of the HIRA complex in depositing H3.3. Ubinuclein 2 (UBN2), which is a

paralog of UBN1 that interacts with HIRA [96], forms with HIRA another complex which appears distinct from the one comprising UBN1 [104]. These two complexes could cooperatively deposit H3.3 onto cis-regulatory regions in mouse embryonic stem cells (mESCs). While yeast exhibits a single Hpc2, understanding why other eukaryotes evolved with the emergence of two UBN paralogs will be interesting to explore. In a manner that compares with H3.3 KO, HIRA is required for proper development in vertebrates, possibly reflecting their tight functional connexion. HIRA KO mice die by stage E10.0 or E11.0 as a consequence of abnormal gastrulation [105].

DAXX was originally described as a Fas death receptor binding protein that induced apoptosis via JNK pathway activation [106]. ATRX was identified through the discovery of mutations in the corresponding gene in a form of X-linked mental retardation (ATR-X syndrome) in young males [107,108]. It is a member of the SNF2 family of chromatin remodeling factors [109]. Chromatin remodelers consist of a group of protein complexes containing an ATPase subunit that regulate a number of DNA transactions by sliding, removing and reconstructing nucleosomes [110]. The discovery of DAXX and ATRX in complex with H3.3 suggested a role for these two proteins in the deposition of this variant [55,56]. Although DAXX and ATRX along with HIRA associate with H3.3, they form distinct H3.3 complexes [111]. Interestingly, in contrast to HIRA, DAXX and ATRX have no known counterparts in budding yeast suggesting a more recent function for this complex possibly in metazoans. In the DAXX-ATRX-H3.3 complex, DAXX is the component that interacts directly with H3.3 while ATRX allows the targeting to heterochromatin [112]. The crystal structure of the histone-binding domain of DAXX bound to the H3.3–H4 dimer revealed the principal determinants of human H3.3 specificity with Ala87 and Gly90 in H3.3. DAXX prefers Gly90 in H3.3 over the hydrophobic Met90 in H3.1 [113,114]. As mentioned above, the UBN1 subunit in the HIRA complex has nearly identical points of contact in the proximity of H3.3 G90 although the mechanism for H3.3 G90 recognition are likely distinct [100]. Of note, in human cells overexpressing CenH3^{CENP-A} the strict selectivity of DAXX-ATRX

for H3.3 is altered and DAXX binds the centromeric CenH3^{CENP-A} leading to its mis-localization. This occurs at sites of active histone turnover and involves an unusual heterotypic tetramer containing CenH3 $^{CENP-A}$ -H4 with H3.3-H4 [66,115-118]. In addition, DAXX can function independently of ATRX to repress endogenous retroviruses, in a process that does not involve H3.3 incorporation into chromatin [119]. Both loss of DAXX and ATRX are embryonic lethal in mice at stage E9.5 [120,121]. ATRX KO cells exhibit loss of the H3K9me3 heterochromatin modification, loss of repression and aberrant allelic expression arguing for a role of ATRX in the maintenance of silencing memory at imprinted loci [122].

2.3.2.3 CenH3^{CENP-A}-H4 Chaperone

The histone chaperone dedicated to the centromeric histone H3 variant CenH3^{CENP-A} is the Holliday junction recognition protein (HJURP) (for review [69]) **(Fig. 2.3)**. Described in 2007 as a protein that binds Holliday junction, HJURP was initially involved in the homologous recombination (HR) pathway in the double strand break (DSB) repair mechanism [123]. As for CAF-1, HIRA and DAXX-ATRX, the biochemical purification of the protein complex associated with CenH3^{CENP-A} in human cells enabled to identify HJURP as a CenH3^{CENP-A} histone chaperone [124,125]. The yeast suppressor of chromosome mis-segregation 3 (Scm3) stands as the HJURP counterpart in *S. Cerevisiae*. Despite their conserved function in CenH3 deposition, HJURP/Scm3 homologues exhibit high degrees of sequence divergence among species likely as a consequence of the rapid co-evolution of the chaperone and the variant. The selectivity of HJURP for CenH3^{CENP-A} is mediated by the interaction of its CENP-A binding domain (CBD) in the N-terminal part of the protein with the CENP-A targeting domain (CATD) in CenH3^{CENP-A} (composed of the α2 helix and the loop L1) [126]. Structural analysis showed that the CBD of HJURP binds a CenH3^{CENP-A}–H4 dimer [127]. The homodimerization of HJURP, through its HJURP C-terminal domain 2 (HCTD2), is required for CenH3^{CENP-A} deposition, leading to the hypothesis that HJURP dimerization allows to bring two CenH3^{CENP-A}–H4 dimers to form the (CenH3^{CENP-A}–H4)$_2$ tetramer at centromeric DNA [128]. Of note, in addition to its role as

chaperone of CenH3^{CENP-A}, HJURP also interacts and recruits CENP-C, another kinetochore component, at centromere [129].

2.3.3 Other H3-H4 Chaperones

Other H3-H4 chaperones, less selective, bind several H3 histones (both replicative and variants) and participate in the nucleosome assembly line. Upstream or downstream the new deposition process, they can be involved in handling soluble new histones or in recycling nucleosomal histones or both.

The anti-silencing function 1 (ASF1) histone chaperone was initially identified in *S. cerevisiae* in a screen for silencing defects upon overexpression [130]. ASF1 was the first histone chaperone crystallized in complex with H3-H4 [131,132]. Its domain interacting with histones contains an Ig-like fold that binds the α2-α3 helices of histone H3. Together with CAF-1, ASF1 facilitates chromatin assembly linked to DNA synthesis *in vitro* [133,134]. However, ASF1 is not directly involved in the deposition mechanism but likely acts by transferring H3-H4 dimers to the downstream histone chaperones that are depositing the new histones H3-H4. In mammals, two paralogous proteins exist, ASF1a and ASF1b with distinct cellular roles [135]. ASF1a and ASF1b co-purified with H3.1 and H3.3 complexes arguing for their role in both DSC and DSI assembly lines [86]. Although ASF1a and ASF1b do not exhibit preferences for H3.1/2 or H3.3 *per se* and can associate with both H3.1/2-H4 and H3.3-H4 dimers, ASF1a harbors a preference for the HIRA complex whereas ASF1b interacts preferentially with CAF-1 [136]. ASF1a and ASF1b bind a motif named B domain which is present in both HIRA and CAF-1p60 subunits but how the interaction preferences are achieved is not fully understood [137]. Importantly, ASF1 a and b interact with the B domain of CAF-1p60 or HIRA through a conserved hydrophobic groove at a site opposite to that of their interaction with H3–H4. A ternary complex (CAF-1–ASF1–H3.1–H4) or (HIRA-ASF1-H3.3-H4) could thus represent an intermediate that enables histones to be handed over from one chaperone to the next. ASF1a and b bind H3-H4 at the tetramerization interface and therefore sterically prevent their tetramerization [131,132,138]. Furthermore, these

chaperones are able to disrupt an $(H3\text{-}H4)_2$ tetramer into two H3-H4 dimers but alone cannot disengage it from DNA. Notably, in addition to participating upstream in the new deposition, ASF1a and b are also involved in old/pre-existing/parental histones recycling during replication in association with mini chromosome maintenance 2 (MCM2) [139,140].

MCM2 is a subunit of the Cdc45-MCM(2-7)-GINS (CMG) replicative helicase that unwinds DNA and separates the two strands of the double helix prior to the action of DNA polymerases [141]. Although its binding capacity to histones was discovered almost 20 years ago [142], its central role in handling both old and newly synthesized histones during replication was highlighted more recently [139]. Together with other subunits of the helicase (MCM3–7), MCM2 coimmunoprecipitates with H3-H4, enriched in parental histones, from nuclear extracts in S phase human cells. In contrast, only MCM2 co-immunoprecipitates with newly synthesized histones H3-H4 and with ASF1a and b in human cytosolic cells extracts. This suggests that independently from its role at the replication fork, MCM2 could also play a specific role as a histone chaperone. Biochemical studies revealed that the N-terminal tail of MCM2, containing the histone-binding domain (HBD), directly binds histone H3 *in vitro* [143]. Then, structural analysis showed that MCM2 HBD can bind both an $(H3\text{-}H4)_2$ tetramer and a dimer of H3-H4 engaged in an interaction with the other chaperone ASF1 [144,145]. The interaction of MCM2 with histones involving a tetramer-to-dimer transition would be important for the proper dynamics of histones during passage of the replication fork. Moreover, the finding that MCM2 can bind all H3 (H3.1, H3.2, H3.3 and CENP-A) [144] suggests that this mechanism to handle histones could apply throughout the entire genome [146].

POLE3 and POLE4 are accessory subunits of the mammalian Polε, the polymerase that is active on the leading strand at the replication fork [147]. A recent study discovered that the human POLE3-POLE4 complex binds to histones H3-H4 (either H3.1 or H3.3) as dimer or tetramer [148]. POLE3-POLE4 binds H3-H4 in the context of chromatin during replication excluding the possibility that it chaperones soluble histones. Moreover, POLE3-POLE4

associates with histones carrying modifications characteristic of both newly synthesized and parental histones suggesting that POLE3-POLE4 may handle both new and old histones in proximity of the leading strand. Another recent study in *S.cerevisiae* showed that the yeast counterparts Dpb3 and Dpb4, drive the recycling of parental histones onto the leading strand, indicating that their function in histone dynamics at replication fork is likely conserved [149]. We will discuss later how the dynamics of recycling and deposition of histones respectively on the leading and lagging strand has combined all these features.

2.4 Deposition of H3-H4 Histones onto DNA

During most of DNA processes such as replication, repair or transcription, the nucleosome organization is disassembled then reassembled. The disassembly is generally required to permit access of the actors of the different machineries to DNA and the reassembly is needed to maintain the chromatin organization to ensure genome integrity. During the reassembly, deposition of both new and old/pre-existing/parental histones occurs. Recycling of old histones contributes to preserve positional information and allows variants and PTMs transmission while new histones deposition could give rise to epigenome fluctuations. The mechanisms of new histone deposition involving histone chaperones have been explored over the last 25 years while those involved in the deposition/recycling of old histones were under investigations more recently. Of note, the studies on histone dynamics *in vivo* benefited in particular from the SNAP-tag technology which allows to visualize selectively either newly synthesized or old histones in the cell [68,150].

2.4.1 Deposition of New H3-H4 Histones

2.4.1.1 New H3.1/2-H4 Deposition by CAF-1

The histone chaperone CAF-1 deposits new replicative histones at sites of DNA synthesis both during replication when DNA is duplicated and during repair when DNA damage is repaired [78,151] **(Fig. 2.4)**. Thus, CAF-1 deposits new H3.1/2 onto DNA in a DSC manner both coupled

to replication during S phase and independently of replication at sites of DNA repair throughout interphase [103,41]. CAF-1 is recruited to sites of DNA synthesis mainly by the interaction of its large subunit p150 with the DNA sliding clamp, PCNA [79,80]. The phosphorylation of CAF-1 p150 by Cdc7/Dbf4 during S phase promotes this interaction by regulating the homo-oligomerization status of p150 [81]. The p60 subunit is also a substrate for phosphorylation by cdk *in vitro*, which may represent another mechanism by which CAF-1 deposition activity is regulated [152,153]. The SNAP-technology enabled to follow the deposition of newly synthesized H3.1 in the cell. H3.1 new deposition colocalized with replication sites during S phase and CAF-1 depletion abrogated this new H3.1 deposition [39]. To date, no other histone chaperone proved able to deposit H3.1 in the absence of CAF-1. However, H3.3 deposition promoted by HIRA does occur at replication sites when CAF-1 is depleted [39]. These findings suggest that, when the assembly coupled to DNA synthesis is defective, the gaps left free could be filled up via in a compensatory mechanism involving the DSI nucleosome assembly pathway in order to maintain chromatin integrity.

2.4.1.2 New H3.3-H4 Deposition by HIRA

The HIRA complex is involved in the DSI deposition of new H3.3 histone variant **(Fig. 2.4)** and this new deposition occurs throughout interphase as visualized in the cell by using the SNAP-tag technology [39]. H3.3 is enriched in the body of transcribed genes, at promoter regions at both active and inactive genes and also at genic and intergenic regulatory regions [55]. Thus, HIRA-dependent enrichment of H3.3 in the coding regions of genes appears mainly associated with active transcription. The deposition of H3.3 at transcribed genes was underscored by the co-immunoprecipitation of the HIRA complex with both the initiating and elongating forms of the RNA pol II harboring specific phosphorylation at serine 5 and serine 2 into its carboxy terminal domain (CTD), respectively [39]. The interactions between the HIRA complex and several actors of the transcriptional process further support the link between HIRA-dependent H3.3 deposition and transcription [154-156]. Furthermore, post-translational modifications of the HIRA subunit

can modulate the H3.3 deposition activity of the complex [157,158].

The HIRA complex can also promote deposition of H3.3 independently of transcription in several circumstances, at UV damage sites where H3.3 deposition occurs upon detection of the DNA damage prior to repair [159], at fertilization in paternal chromatin before the first round of DNA replication [52,53] and onto viral DNA upon virus infection. In this latter case, depending of the system, H3.3 accumulation onto viral DNA correlated with active or repress viral transcription and with virus latency [160-162].

The HIRA complex shows unique DNA binding properties as compared to other H3-H4 histone chaperones, and its depletion increases DNA sensitivity to nucleases [39]. The HIRA complex from cell extracts binds to both double-stranded and single-stranded DNA suggesting that it could recognize particular DNA structures [103]. The ability of the HIRA complex to bind naked DNA provides a mechanism of new H3.3 deposition that may operate to avoid nucleosome-free DNA regions which could be deleterious for maintenance of chromatin organization and genome integrity. This leads to the proposal of a nucleosome gap filling mechanism for the HIRA-dependent H3.3 deposition and a crucial role to maintain chromatin integrity [39,163].

2.4.1.3 New H3.3-H4 Deposition by DAXX-ATRX

The histone chaperone DAXX-ATRX is responsible for the enrichment of H3.3 in heterochromatin at pericentric regions and telomeres [55,56]. Although not formally demonstrated, DAXX-ATRX is assumed to be key for the deposition of new histone variant H3.3 at these heterochromatic regions in a DSI manner **(Fig. 2.4)**. Whether the deposition of H3.3 by DAXX-ATRX at these specific locations occurs during a particular time window during the cell cycle and whether it does link to the deposition of H2AZ variant will be interesting to explore [164]. While DAXX directly interacts with H3.3 [113,114], ATRX recognizes H3K9me3 through its ATRX-Dnmt3-Dnmt3L (ADD) domain and could therefore target DAXX to these locations [112]. DAXX-ATRX also mediates H3.3 deposition at G-quadruplexes (G4) and at endogenous

retroviruses through a possible direct binding of ATRX to these structures and transposable elements [165,166]. These data have led to propose a role for ATRX in suppressing recombination at telomeric repeats by resolving G4 structures through the deposition of H3.3 [167]. At transposable elements, the incorporation of H3.3 is proposed to silence repetitive elements through regulation of H3K9me3 [168,166].

DAXX-ATRX also mediates H3.3 enrichment outside of heterochromatin and repetitive elements. DAXX-ATRX together with the HIRA complex was proposed to induce virus latency by enabling H3.3 accumulation onto viral DNA [160]. In the nervous system, the serine residue 669 of DAXX is phosphorylated by the homeodomain-interacting protein kinase 1 (HIPK1) and upon neuronal activation, the calcium-dependent phosphatase calcineurin (CaN) dephosphorylates S669 [169]. This dephosphorylation, by enhancing DAXX activity, increased H3.3 enrichment at promoters and enhancers of immediate early genes leading to their active transcription. However, a major role for the H3.3-mediated activity of DAXX-ATRX is likely linked to its impact on heterochomatin function as shown above.

2.4.1.4 New CenH3^{CENP-A}-H4 Deposition by HJURP

The histone chaperone HJURP deposits the new histone variant CenH3^{CENP-A} at centromere in a DSI manner during late mitosis (telophase)/early G1 in mammals **(Fig. 2.4)** [68,124,125]. HJURP localizes to centromeres at the time of CenH3^{CENP-A} deposition and CDK kinases control its timely recruitment to centromeres in late mitosis by changing its phosphorylation status [170,171]. HJURP interacts with DNA through a specialized domain, which is essential to deposit CenH3^{CENP-A} at centromeres, highlighting that HJURP is not merely escorting CenH3^{CENP-A}, but plays an active part in CenH3^{CENP-A} deposition [170]. Of note, although HJURP is the histone chaperone involved in the final step of CenH3^{CENP-A} deposition, numbers of other factors are required for the proper incorporation of this histone variant into centromere (review [69]). CenH3^{CENP-A} post-translational modifications are important for its deposition. Ser68 phosphorylation of CenH3^{CENP-A} prevents an interaction with HJURP in the pre-deposition

complex, helping to prevent premature loading [172]. The crystal structure at the interface of CenH3^{CENP-A} and HJURP shows that Ser68 lies in the histone variant binding domain of HJURP. Moreover, Lys124 ubiquitylation of CenH3^{CENP-A} plays a role in CenH3^{CENP-A} deposition by controlling the stability of the CenH3^{CENPA}–HJURP complex through a regulatory mechanism involving the cullin 4 (CUL4) ubiquitin ligase [173]. The new deposition of CenH3^{CENP-A} only occurs in late mitosis/early G1 phases, thus CenH3^{CENP-A} is diluted during S phase and distributed evenly to both daughter chromosomes in mammals [68]. In addition to deposit new CenH3^{CENP-A}, HJURP may be also required for its retention during S phase. By interacting with the replicative helicase complex, HJURP is proposed to retain and recycle CenH3^{CENP-A} following DNA replication [174]. During S phase, new deposition of both H3.1 (in a DSC manner) and H3.3 (through a possible post-replicative gap-filling mechanism) is observed, filling the gaps generated by diluting CenH3^{CENP-A}. Interestingly, the detected loss of H3.3 only later in G1 phase argues that H3.3 variant could serve as a CenH3^{CENP-A} placeholder [175].

2.4.2 Recycling of Old H3-H4 Histones

Chromatin integrity is critical for cell function and identity but is challenged by DNA processes that involve nucleosome disassembly. How chromatin architecture and the information that it conveys are preserved? For example, during replication, the chromatin structure is affected by the transient disruption of histone-DNA interaction from old/pre-existing/parental nucleosomes located ahead of replication forks (Disassembly). Chromatin assembly onto daughter strands relies on two distinct processes: the transfer of old histones (Recycling) and second the deposition of new histones (New deposition). The latter process, as mentioned before is regulated by the CAF-1 complex that deposits new H3.1/2-H4 histones onto both daughter strands. Experiments in the 1980s with bulk chromatin demonstrated the retention of parental histones on daughter strands [176,177]. The recycling of old histones with their PTMs and the subsequent modifications of new histones to mirror the parental ones would participate in the maintenance of chromatin identity. The transmission of parental PTMs during replication

appears to occur in human cells [178] This is critical for features to be inherited from one cell to the next.

ASF1 has been the first H3-H4 chaperone implicated in the recycling of old histones during replication [139]. ASF1 was proposed to handle old histones at replication fork via an ASF1-(H3-H4)-MCM2-7 intermediate. An important role for MCM2 emerged based on structural analysis showing that MCM2, in contrast to ASF1, can bind an $(H3\text{-}H4)_2$ tetramer [144,145]. This mode of binding implies that, once evicted from DNA possibly by the force of the helicase and the activity of remodelling factors, nucleosomal H3-H4 could be directly transferred to MCM2 as a tetramer. After this step old tetrameric $(H3\text{-}H4)_2$ could simply be directly loaded onto the newly synthesized DNA. Alternatively, old H3-H4 could be deposited as dimers after splitting by ASF1 [146]. Although reassociation of the two parental dimer partners might be favored most of the time, mixing H3.3-H4 dimers, but not H3.1-H4 dimers, was reported with potential important role in the inheritance of epigenetic traits [179,180].

During replication, the two daughter chromatids differ in how they are replicated. The leading strand synthesis occurs in the direction of the fork progression while the lagging strand proceeds in interspersed segments in an opposite direction. Recycling old histones on leading and lagging strands exploit distinct mechanisms involving histones chaperones. In yeast, while MCM2 operates on the lagging strand, Dpb3-Dpb4 subunits of the polymerase ε act on the leading strand [149,181]. The function of MCM2 in recycling old histones onto the lagging strand is conserved in human [182]. POLE3-POLE4, the human counterpart of Dpb3-Dpb4, recently described as a H3-H4 chaperone whose depletion affects chromatin at replication fork, could similarly participate in the recycling of old histones onto the leading strand in human [148]. Whether, the evicted $(H3\text{-}H4)_2$ tetramer splits in to dimers before recycling/deposition onto the leading strand remains to be explored, in particular in light of possible connection with Asf1. A scheme of the current model for histone dynamics at replication fork, involving old histone recycling and new histone deposition, is shown in **Fig. 2.5**.

Of note in yeast, Cohesion establishment factor 4 (Ctf4), a replisome factor that links the CMG helicase on the leading strand to the DNA polymerase α on the lagging strand (through a direct interaction with both GINS and polα) [183], participates in the recycling of old histones on this latter strand [181]. Ctf4 and its human counterpart, Acidic nucleoplasmic DNA binding protein-1 (And-1), form homotrimers that exhibit homology with the homo-trimeric form of the HIRA subunit of the histone chaperone complex HIRA [103]. This intriguing homology could suggest for Ctf4/And-1 and HIRA a similar way of mediating protein and DNA interactions at particular bubble DNA structures as encountered at replication fork for Ctf4/And-1 and perhaps at transcription sites for HIRA.

The existence of distinct mechanisms for recycling old histones on the two daughter strands raises new interesting hypothesis [184]. Indeed, while ensuring an equal partitioning of old histones for most cells, it may as well offer an opportunity for unequal partitioning. For example, in *Drosophila* male germline and adult midgut, replicative H3 and CenH3^{CENP-A} are asymmetrically distributed, respectively. The daughter stem cell retains the parental/old histones while the post-mitotic differentiating daughter cell genome is assembled with new histones [185,186]. The existence of distinct mechanisms to recycle old histones might be a way to regulate asymmetric distribution of old histones onto the two daughter cells in the wake of the replication. This could be crucial to initiate a differentiation program by loosing parental marks.

2.5 Concluding Remarks and perspectives

Chromatin can protect DNA from various deleterious threats while remaining flexible to enable the regulation of gene expression and programmed changes in cell identity to occur during normal development. The histone H3 family and its various chaperones are crucial for allowing dynamic accessibility to particular genomic loci. Over recent decades, much progress has been made in the study of histones and their modes of incorporation into chromatin. This is

particularly true for histones H3-H4 with the identification of histone chaperones that are often specialized in the deposition of one peculiar H3 variant at a particular time and at specific locations onto the genome. We are unveiling complete histone H3 deposition pathways from their site of synthesis to their sites of delivery and we are also now currently elucidating how old histones are recycled at replication fork. Open questions remain regarding this network of histones and histone chaperones. For example, histone H3 chaperones are most often protein complexes whose stoichiometry, post-translational modifications and functional regulations are still poorly characterized and understanding how they may link to cell cycle control and cell fate will be extremely exciting. While the deposition process of H3-H4 is now rather well understood, the mechanisms and histone chaperones involved in the deposition of H2A-H2B dimers still needs to be deepened. Processes of old histone recycling started recently to be deciphered at replication fork but old histone recycling also occurs at DNA repair and transcription sites [187,188]. Most fascinating is to understand how the marking with particular variants actually experience cell division and can be restored after the passage of the replication fork. Elucidating therefore the mechanisms that operate during repair and transcription would also be crucial. Furthermore, exploring how this network of histones and histone chaperones is potentially rewired when one of several of the actors are mutated or deregulated in particular during cancer will bring undoubtedly important new findings in the field.

Figure Legends

Fig. 2.1: Differences between replicative histones (H3.1 and H3.2) and histone variants (H3.3 and CenH3^{CENP-A}) in human. Replicative histone genes are organized in clusters and lack introns whereas histone variants are encoded by single genes (two for H3.3 and one for CenH3^{CENP-A}) and have introns. While H3.3A and H3.3B genes encode the same protein, their architecture is different. In particular, their promoter regions contain distinct putative binding sites for transcriptional regulators. Transcripts of replicative histones do not undergo polyadenylation and harbor a 3' stem-loop structure while transcripts of histone variants are polyadenylated and processed through splicing like most RNA pol II mRNAs. Replicative histone genes are highly transcribed during S phase which is not the case of histone variant genes that can be transcribed with various timings depending of the variant (throughout the cell cycle for H3.3 and during G2/M phases for CenH3^{CENP-A}) .

Fig. 2.2: (A) Alignment of human amino acid sequences corresponding to the replicative histones H3.1 and H3.2 and the histone variants H3.3 and CenH3^{CENP-A}. Sequences are compared to H3.1 and the residue differences are highlighted. H3.1 and H3.2 differ by only one residue at position 96. H3.3 differs from H3.1 by five residues (at positions 31, 87, 89, 90 and 96) and from H3.2 by four residues (at positions 31, 87, 89 and 90), while the amino acid sequence of CenH3^{CENP-A} exhibits less than 50% identity with H3.1. The histone fold domain containing three α-helices and two loops is shown. **(B)** Crystal structures of H3.1, H3.3 and CenH3^{CENP-A} nucleosome core particles (NCP). The NCP contains an histone octamer that consists of a tetramer with two H3-H4 dimers ((H3-H4)$_2$) flanked by two H2A-H2B dimers. Histone octamer from both H3.1 and H3.3 NCPs is wrapped by 147 bp of DNA [57] whereas 121 bp of DNA wrapped the histone octamer from CenH3^{CENP-A} NCP [65].

Fig. 2.3: Replicative histones (H3.1 and H3.2) and histone variants (H3.3 and CenH3^{CENP-A}) are *de novo* deposited onto DNA by their dedicated chaperones using two different nucleosome assembly pathways, DNA synthesis coupled (DSC) and DNA synthesis independent (DSI), respectively. H3.1/2-H4 and CenH3^{CENP-A}-H4 dimers bind to one histone chaperone, CAF-1 and HJURP, respectively, while H3.3-H4 can associate with two distinct histone chaperones, the HIRA complex and DAXX-ATRX. Of note, the homo-oligomerization status of each component is not indicated and only one molecule is represented.

Fig. 2.4: (A) Enrichment of H3.1, H3.3 and CenH3^{CENP-A} mediated by their dedicated histone chaperones at specific genomic sites and/or during particular DNA processes in cycling cells. CAF-1 deposits replicative H3.1/2-H4 genome wide mainly during replication but also during DNA repair. The HIRA complex deposits H3.3-H4 at active genes, promoters, sites of DNA repair and potentially at any transient nucleosome free region by a gap-filling mechanism, while DAXX-ATRX is involved in the enrichment of H3.3-H4 mainly at heterochromatin (telomere and pericentromere) but also at regularory elements. HJURP mediates the incorporation of CenH3^{CENP-A} –H4 at centromere (in centric heterochromatin). **(B)** Genomic distribution of H3.1, H3.3 and CenH3^{CENP-A} [20] from published ChIP-Seq data in HeLa cells [140,66]. The plot shows the enrichment relative to input for all variants at a representative region spanning the centromere and the proximal short and long arms of chromosome 18 (p11.21-q21.1). Enriched regions are highlighted in darker colors, illustrating the partitioning of the genome into chromatin domains associated with specific histone H3.

Fig. 2.5: Current model of histone dynamics at the replication fork. **(A)** For each old/parental nucleosome disrupted by the replication fork passage, a H3-H4 tetramer is available (Disassembly). The old H3-H4 histones are recycled on newly synthesized DNA either directly as a tetramer or potentially as two dimers (Recycling). New H3-H4 dimers are deposited onto

newly synthesized DNA to ensure a full complement of nucleosomes on the nascent DNA (New Deposition). Recycling of old/parental histones and deposition of new histones are thought to occur randomly on both the leading (in orange) and the lagging strand (in green). **(B)** The mechanisms and the histone chaperones involved in the recycling of old histones on the leading and the lagging strands are distinct. At the replication fork, the CMG (Cdc45-MCM(2-7)-GINS) helicase on the leading strand unwinds the DNA. The homotrimer And-1/Ctf4 links the helicase on the leading strand to the polα on the lagging strand through its interaction with both GINS and polα. While the two accessory subunits of polε (POLE3 and POLE4) mediate the recycling of old H3-H4 histones on the leading strand, the helicase subunit, MCM2, operates on the lagging strand. It is still not fully understood whether H3-H4 are directly recycled as tetramers or whether they split as dimers before deposition or whether both events happen. Moreover, if H3-H4 split, whether ASF1 handles the H3-H4 dimers before deposition remains unclear. **(C)** To fulfill the requirement for nucleosome assembly, deposition of newly synthesized H3.1/2-H4 dimers occurs on both strands by the histone chaperone complex CAF-1 through its interaction with the sliding clamp PCNA. The histone chaperone ASF1 would hand over H3.1/2-H4 dimers to CAF-1 before deposition.

Table 2.1 Histone H3-H4 Chaperones

Chaperone	Human subunit(s)	S.cerevisiae subunit(s)	Histone preference(s) in human	Function(s)
CAF-1 complex	p150 (CHAF-1) p60 (CHAF-2) p48 (RbAp48)	cac1 cac2 cac3	H3.1/2-H4	New deposition DSC
HIRA complex	HIRA CABIN1 UBN1 or UBN2	Hir1p and Hir2p Hir3p Hpc2p	H3.3-H4	New deposition DSI
DAXX-ATRX	DAXX ATRX	NA	H3.3-H4	New deposition DSI
HJURP	HJURP	Scm3	$CenH3^{CENP-A}$	New deposition DSI Recycling ?
ASF1	ASF1a or ASF1b	Asf1	H3.1/2-H4 H3.3-H4 $CenH3^{CENP-A}$?	Transit, Buffer, Handover, Recycling
MCM2	MCM2	Mcm2	H3.1/2-H4 H3.3-H4 $CenH3^{CENP-A}$	Recycling
Polε	POLE3 POLE4	Dpb3 Dpb4	H3-H4	Recycling

Not available (NA); DNA synthesis coupled (DSC); DNA synthesis independent (DSI); Chromatin assembly factor 1 (CAF-1); Histone regulator A (HIRA); Histone regulation (Hir); Histone periodic control 2 (Hpc2p); Death domain-associated protein 6 - α-thalassaemia/mental retardation syndrome X-linked (DAXX-ATRX); Holliday junction recognition protein (HJURP); Suppressor of chromosome mis-segregation 3 (Scm3); Anti-silencing function 1 (ASF1); Mini chromosome maintenance 2 (MCM2); DNA polymerase ε (Polε).

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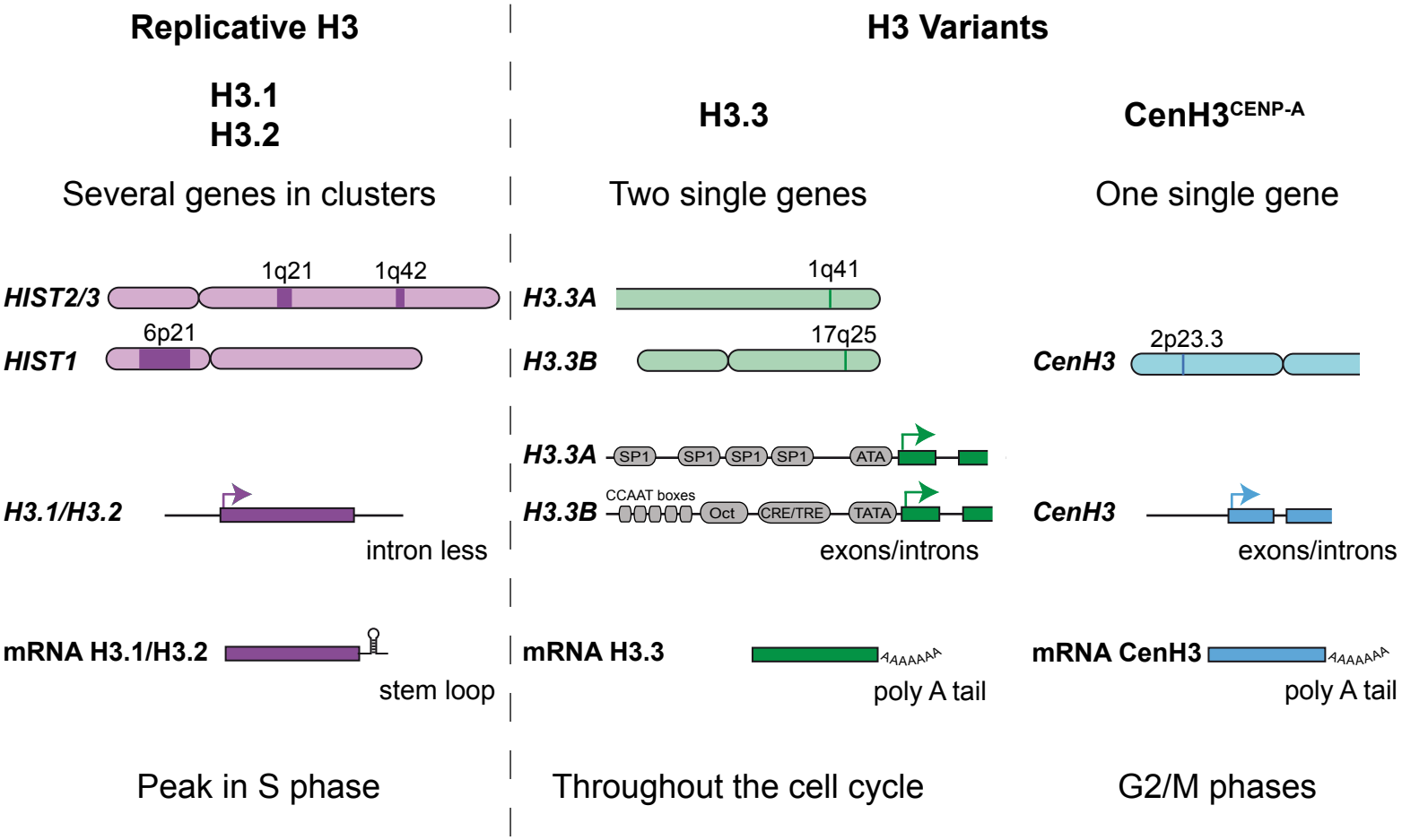
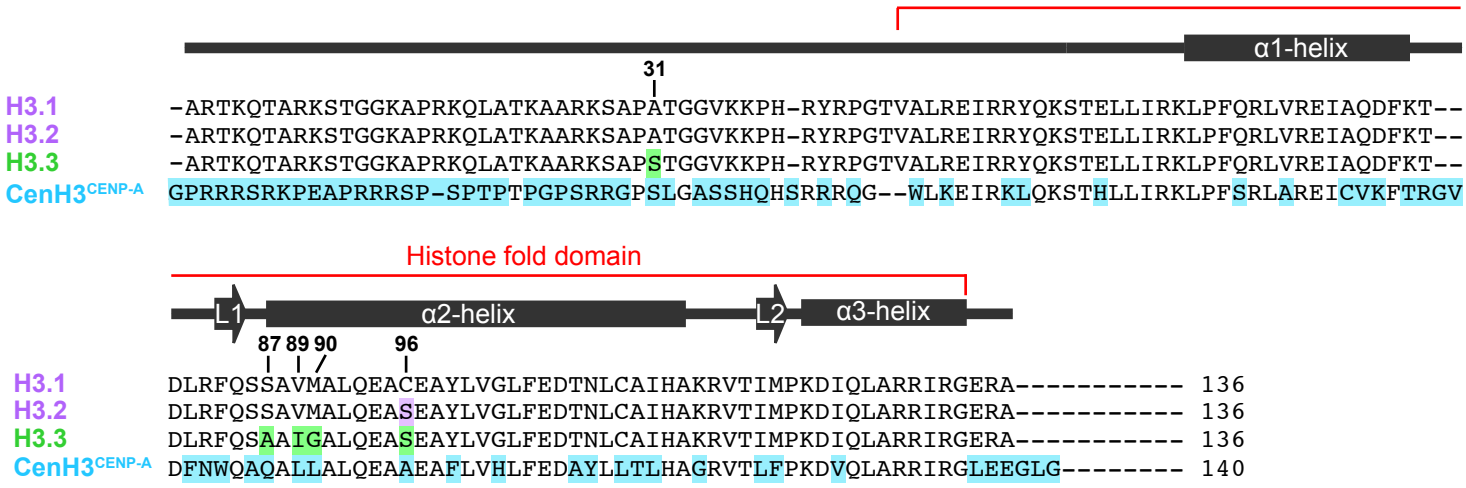
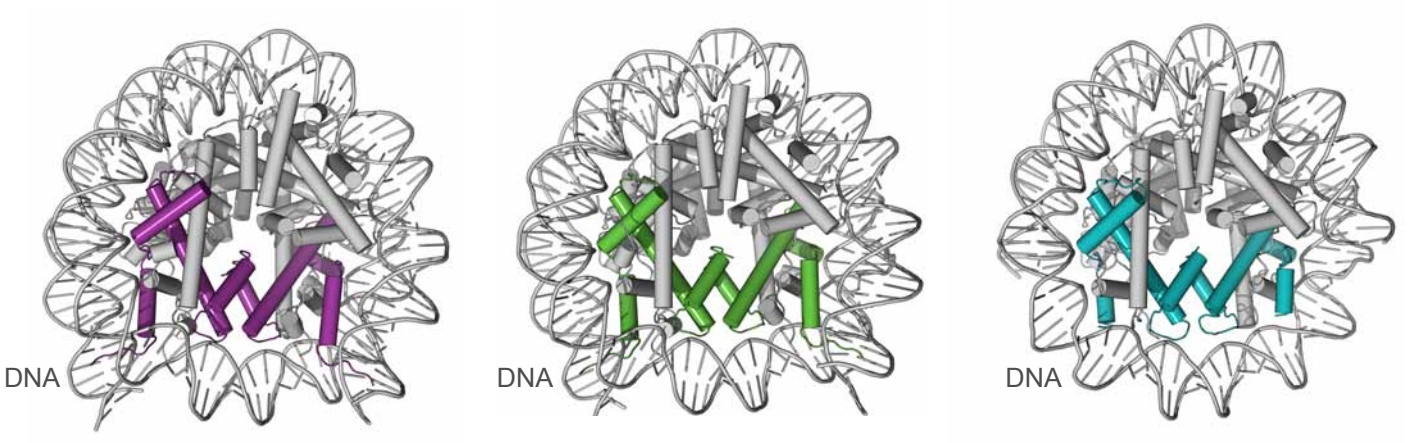
Genomic organization, mRNA structure and temporal expression of H3.1/H3.2, H3.3 and CenH3^{CENP-A}



A Alignment of human H3.1, H3.2, H3.3 and CenH3^{CENP-A} amino acid sequences



B Crystal structures of H3.1, H3.3 and CenH3^{CENP-A} nucleosome core particles



H3.1 core particle (**147** bp of DNA)

H3.3 core particle (**147** bp of DNA)

CenH3^{CENP-A} core particle (121 bp of DNA)

Fig. 2.3

New deposition of H3.1/2, H3.3 and CenH3^{CENP-A} by their dedicated histone chaperones

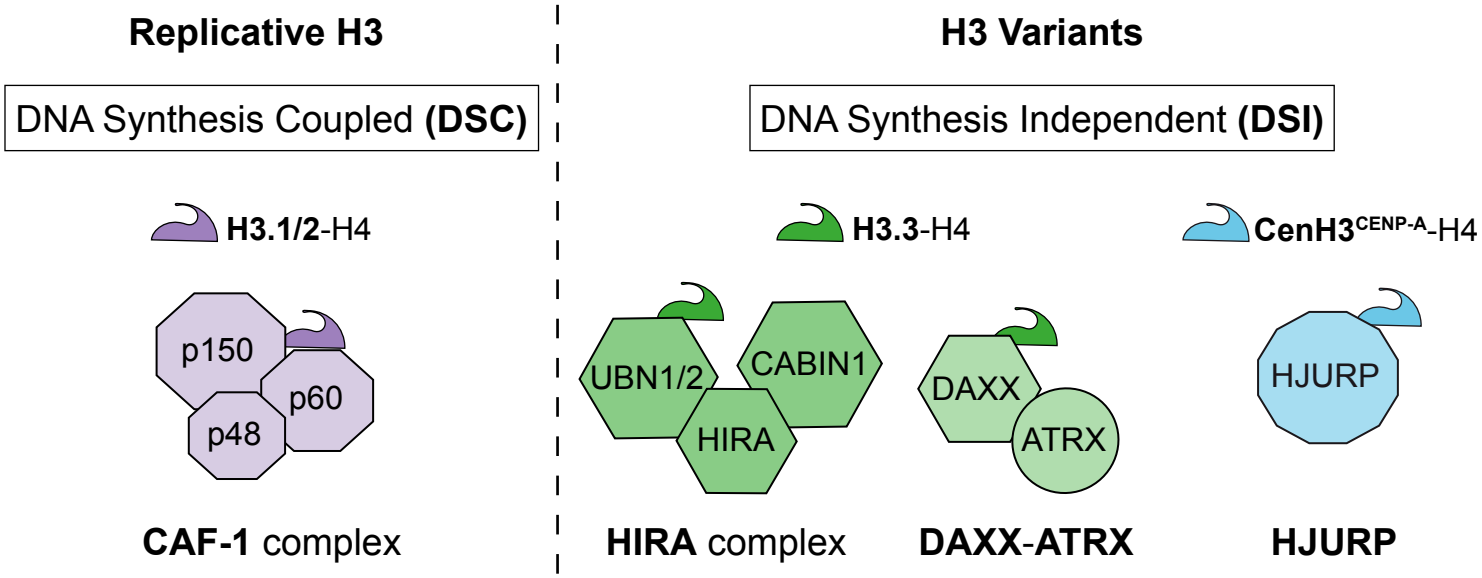
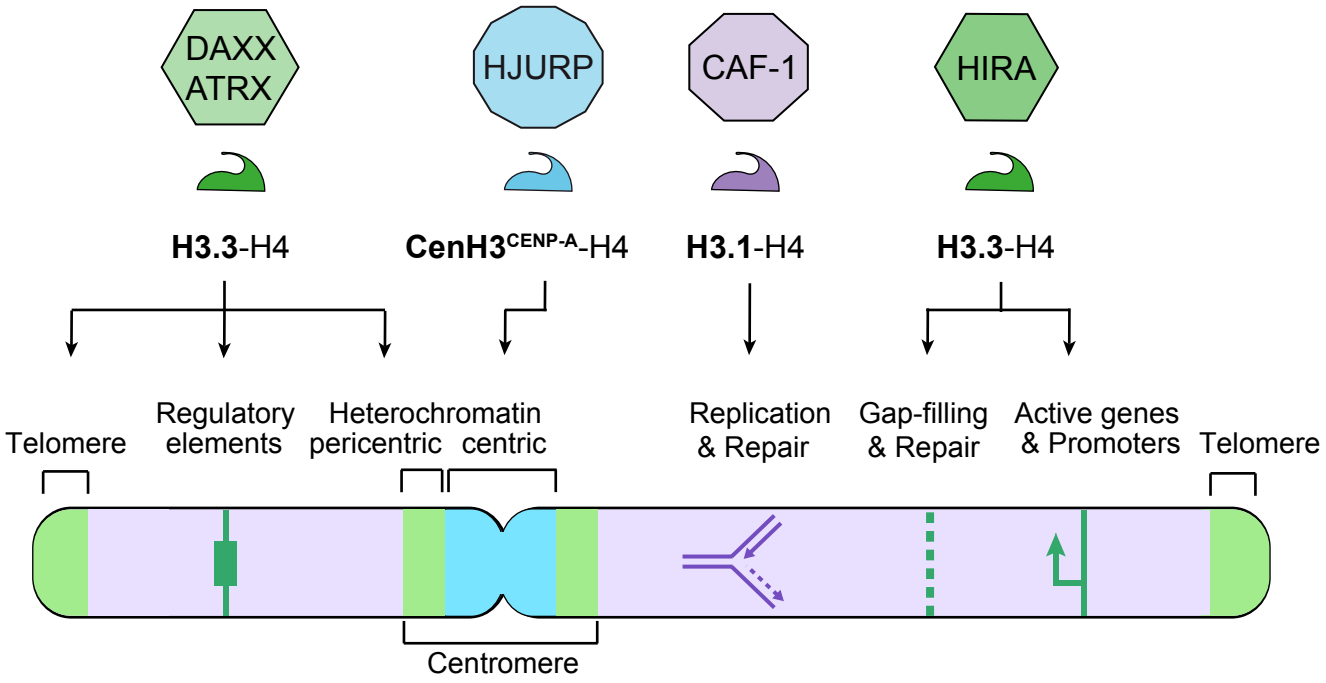


Fig. 2.4

A **Genomic enrichment of H3.1, H3.3 and CenH3^{CENP-A} mediated by their dedicated chaperones**



B **Genomic distribution of H3.1, H3.3 and CenH3^{CENP-A} from ChIP-Seq data in human cells**

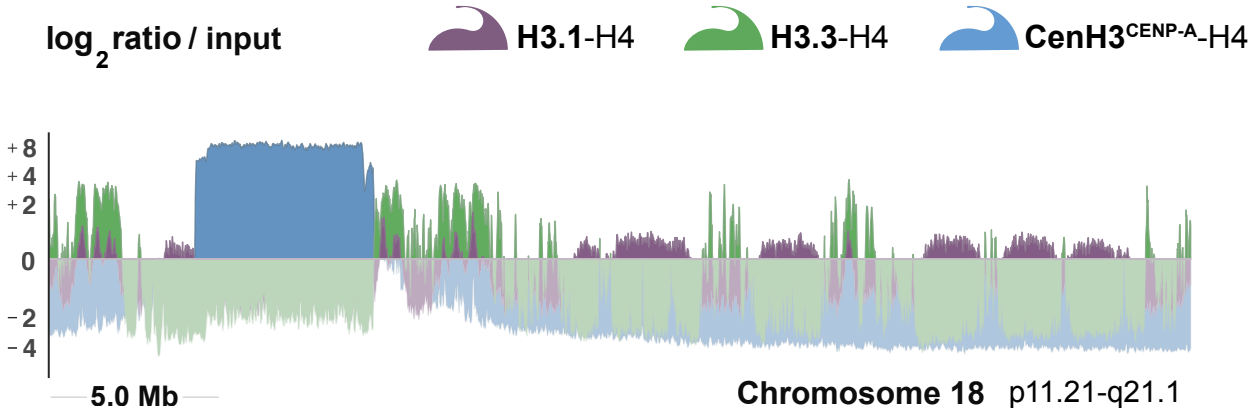
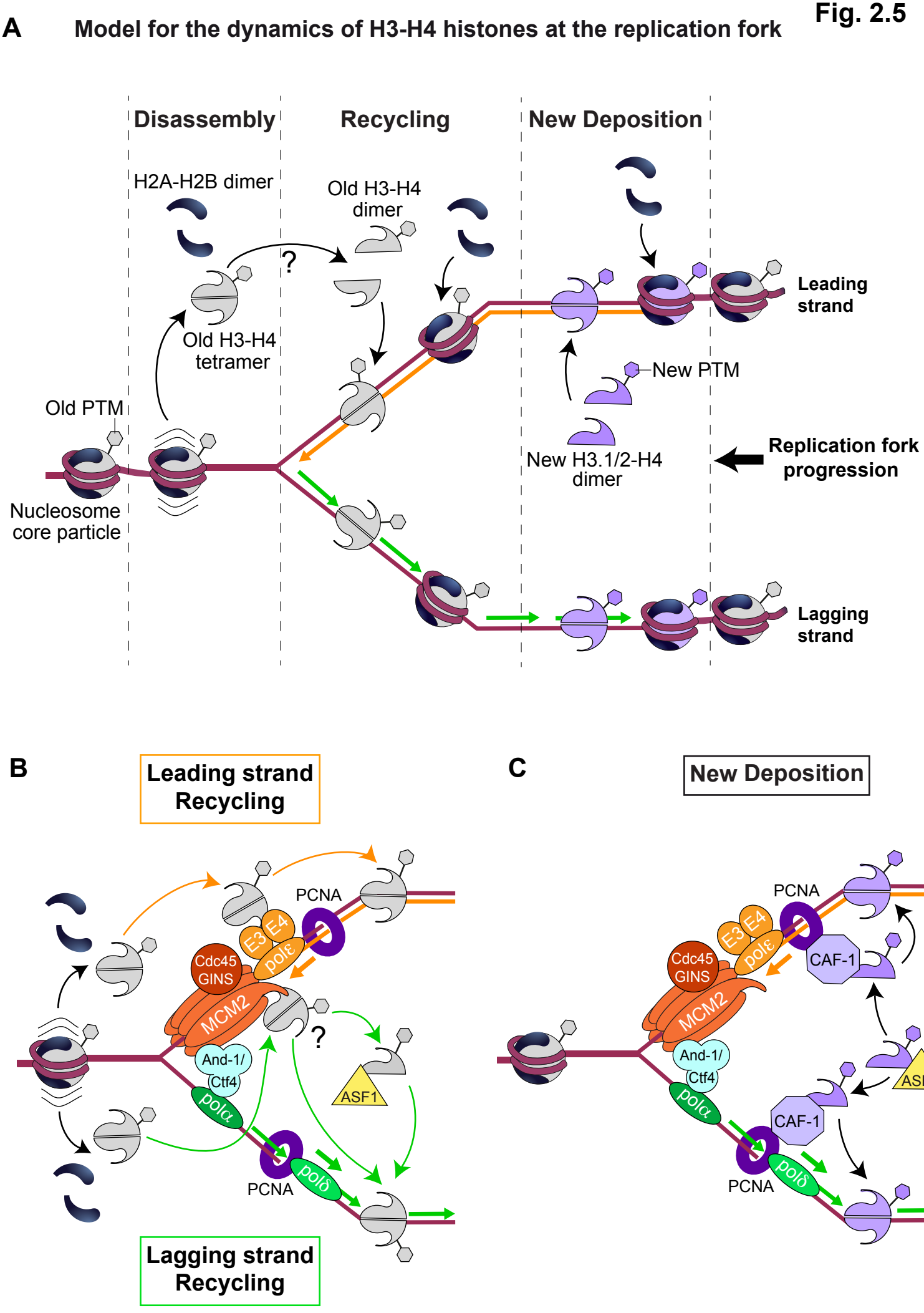


Fig. 2.5


A
Model for the dynamics of H3-H4 histones at the replication fork
Disassembly
Recycling
New Deposition
H2A-H2B dimer
Old H3-H4 dimer
?
Old H3-H4 tetramer
Old PTM
Nucleosome core particle
Leading strand
New PTM
New H3.1/2-H4 dimer
Replication fork progression
Lagging strand
B
Leading strand Recycling
PCNA
E3 E4
polε
Cdc45 GINS
MCM2
And-1/ Ctf4
polα
?
ASF1
PCNA
polδ
Lagging strand Recycling
C
New Deposition
PCNA
E3 E4
polε
CAF-1
Cdc45 GINS
MCM2
And-1/ Ctf4
polα
ASF1
CAF-1
PCNA
polδ