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Mitotic inheritance of genetic and epigenetic information via the histone H3.1 variant

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Summary

The replication-dependent histone H3.1 variant, ubiquitous in multicellular eukaryotes, has been proposed to play key roles during chromatin replication due to its unique expression pattern restricted to the S phase of the cell cycle. Here, we describe recent discoveries in plants regarding molecular mechanisms and cellular pathways involving H3.1 that contribute to the maintenance of genomic and epigenomic information. First, we highlight new advances concerning the contribution of the histone chaperone CAF-1 and the TSK-H3.1 DNA repair pathway in preventing genomic instability during replication. We then summarize the evidence connecting H3.1 to specific roles required for the mitotic inheritance of epigenetic states. Finally, we discuss the recent identification of a specific interaction between H3.1 and DNA polymerase epsilon and its functional implications.

Keywords

Epigenetics; chromatin; DNA replication; histone modifications; histone variants; Arabidopsis

Introduction

In eukaryotes, DNA replication is accompanied by the faithful transmission of complex epigenetic states across the genome. The process of chromatin replication thus implicates two different but related goals—the maintenance of genetic and epigenetic information—that are both required for cellular homeostasis. As these two processes are achieved in the same temporal and spatial window during the cell cycle, they likely rely on many common factors expressed uniquely at the time of chromatin replication.

Among them, the replication-dependent histone H3.1 variant is thought to play a central role in the inheritance of genetic information and the different epigenetic states [1]. In multicellular eukaryotes, H3.1 is specifically expressed during the S phase, in contrast to replication-independent H3.3 which remains available throughout the cell cycle [2,3]. A

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burst of histone expression is needed during DNA synthesis to prevent nucleosome dilution on replicated DNA. However, why multicellular eukaryotes rely on a specific H3 variant during chromatin replication has remained a longstanding question in the chromatin field. The difficulty in answering this question lies with the high level of sequence similarity between H3.1 and H3.3 proteins across all organisms, with only a few amino acids distinguishing these two variants. Recent work using *Arabidopsis thaliana* as a model system has started to shed light on the different molecular mechanisms by which H3.1 participates in the mitotic inheritance of genetic and epigenetic information, which we summarize in this review.

Maintenance of genomic stability by the H3.1 variant

One of the challenges in uncovering specific roles for replication-dependent H3 variants is linked to the fact that these proteins, like other histones, are encoded by large gene families in most biological systems (e.g., five *H3.1* genes in Arabidopsis, and 13 *H3.1*/*H3.2* genes in humans [2,4]). This high gene copy number makes it difficult to completely inactivate H3.1 and study the resulting phenotypes. While the recent establishment of gene editing systems now allows to easily knockout or alter histone gene functions [5–10], early research on H3.1 partially bypassed the problem by inactivating the histone chaperone CAF-1. The heterotrimeric CAF-1 complex is responsible for loading H3.1 on chromatin during replication [11,12], and its inactivation results in the insertion of H3.3 instead of H3.1 in proliferative cells using a gap-filling mechanism (Figure 1a–b) [12–14]. Interestingly, CAF-1 mutants are lethal in many metazoan systems [15,16], but not in Arabidopsis [17], thus making plants a unique model system to study H3.1 via CAF-1 inactivation. Recent work taking advantage of viable CAF-1 null mutants in plants has uncovered a key role for the H3.1 chaperone in mediating genomic stability in Arabidopsis [18]. The absence of CAF-1 function leads to large tandem duplications (~50 to ~1500 kb) and deletion of rRNA gene copies (Figure 1e). Interestingly, tandem duplications in euchromatic regions led to higher transcript levels for the duplicated protein-coding genes, thus establishing one mechanism by which genomic instability can potentially affect phenotypic expression [18].

Evidence suggests that the role of CAF-1 in the maintenance of genome stability depends on its ability to load H3.1 on chromatin during replication [19,20]. The DNA repair protein TONSOKU (TSK/BRUSHY/MGOUN3, also known as TONSOKU-LIKE/TONSL in metazoans) plays a crucial role in resolving stalled or broken replication forks [21–24]. Recently, Arabidopsis TSK was shown to use its conserved tetratricopeptide repeat domain to specifically bind H3.1 by recognizing one residue (alanine 31) that varies in H3.3 [19]. In plants, the TSK-H3.1 interaction is abrogated by mono-methylation at H3.1K27 (H3.1K27me1) [19], a mark deposited by the H3.1 mono-methyltransferases ATXR5 and ATXR6 (ATXR5/6) [25,26], with ATXR6 playing the larger role [27]. As almost all H3.1 proteins newly inserted on chromatin are rapidly mono-methylated by ATXR5/6 during replication [13], the interaction between TSK and H3.1 on chromatin is potentially confined to a short temporal and spatial window, at or near replication forks (Figure 1a). This chromatin-based mode of regulation would be hypothesized to restrict the activity of TSK to resolving stalled or broken replication forks, a seemingly critical regulatory step as depletion of H3.1K27me1 (e.g., in *atxr5/6* mutants) induces genomic instability in the form

of heterochromatin amplification [28], most likely due to ectopic TSK activity (Figure 1c and e) [19]. Interestingly, the reverse situation (i.e., the absence of TSK activity) may also produce genome stability defects (Figure 1d), as shown from sensitivity of *tsk* mutants to the genotoxic drug methyl methanesulfonate [19]. As reduced levels of TSK activity would be expected in a CAF-1 mutant due to the inability to load H3.1 variants on chromatin during replication, it is tempting to speculate that the large tandem duplications and rRNA deletions observed in the absence of CAF-1 are due to TSK-mediated resolution of impaired replication forks not being functional in this mutant background (Figure 1b and e). Overall, these studies strongly suggest a key role played by CAF-1 in recruiting the TSK-H3.1K27 DNA repair pathway to maintain genetic information during replication in plants.

H3.1 and the mitotic inheritance of epigenetic states

Aside from a role for the H3.1 variant in protecting the genome, multiple studies have hinted at functions for H3.1 in the correct transmission of epigenetic states during replication. For example, studies of CAF-1 mutants in Arabidopsis revealed various molecular and developmental phenotypes (e.g., defects in shoot and root apical meristem, and partial loss of heterochromatin silencing and organization) [17,29–32], thus arguing for epigenetic roles for H3.1 that are specific to this H3 variant. Previous work in mammalian systems has shown that some histone post-translational modifications (PTMs) are present on soluble (i.e., pre-deposition) H3.3, while soluble H3.1 proteins are mostly devoid of PTMs [33]. Thus, in the absence of CAF-1, deposition of modified H3.3 during DNA replication could affect the inheritance of epigenetic states.

The H3.1 variant was shown to be involved in the inheritance of the cell-identity marker H3K27me3 during replication [13]. The requirement for H3.1 to maintain this histone mark was proposed to depend on the mono-methyltransferase activity of ATXR5/6. In this model, H3.1K27me1 is a prerequisite for di- and tri-methylation by PRC2, which occurs during DNA replication [13]. Inheritance of epigenetic states may therefore be influenced by differential modifications on H3 variants after their deposition on chromatin, which is when ATXR5/6 are thought to be active on H3.1 based on their catalytic preference for nucleosomal substrates [34].

Other effects of H3.1 on epigenetic inheritance may be related to its interaction with TSK. The initial characterization of TSK indicated clear roles in regulating development and the maintenance of epigenetic silencing at heterochromatic loci [35,36]. More recent work has demonstrated that TSK participates in heat stress priming [37], an epigenetic phenomenon by which previous exposure to heat stress induces a molecular memory that makes plants more resistant to subsequent exposure to the same abiotic stress [38]. A possible model to explain all these results may be that, similarly to CAF-1, TSK directly participates in the deposition of the H3.1 variant during replication. Contradicting this model is the observation that morphological phenotypes between *tsk* and CAF-1 mutants vary significantly. An alternative function for TSK may be that by specifically binding to H3.1 (and not H3.3), it protects the N-terminal tail of H3.1 against spurious interactions during chromatin replication that may affect epigenetic inheritance. Although many pieces of the puzzle appear to be in place, more work is still needed to precisely understand

the overarching mechanism by which H3.1 controls epigenetic inheritance during DNA replication.

Interplay between DNA polymerases and H3.1 during chromatin replication

The recent identification of TSK/TONSL as an H3.1 reader strongly suggests that other proteins may have a similar ability to specifically discriminate H3 variants [1]. Very recently, work in Arabidopsis revealed that the largest catalytic subunit (POL2A) of DNA polymerase epsilon (Pol ε), which catalyzes the synthesis of the leading strand, also binds specifically to H3.1 in the context of an H3.1-H4 dimer/tetramer, an activity that is conserved in POLE1, the mammalian ortholog of POL2A (Figure 2) [39]. A C-terminal CW-type zinc finger domain in POL2A recognizes H3.1 by interacting with amino acid residues A31 and S87, which both vary in H3.3 (T31 and H87). The interaction between POL2A and H3.1 was shown to be required for mediating heterochromatin condensation during meiosis in Arabidopsis [39].

In the last few years, studies using new sequencing-based techniques to probe parental histone segregation during chromatin replication have uncovered specific roles for DNA polymerases and associated complexes. For example, the subunits POLE3 and POLE4 of mammalian POL ε were shown to mediate H3-H4 parental histone segregation to the leading strand [40,41], while MCM2 (a subunit of the replicative CMG helicase) and POLA1 (the catalytic subunit of DNA polymerase alpha) are involved in transferring parental H3-H4 to the lagging strand [40,42,43]. POLA1 was even shown recently to participate in the mitotic recycling of parental H2A-H2B to the lagging strand [44]. These results demonstrate a direct role for the DNA replication machinery in the inheritance of epigenetic states.

The discovery that POL2A specifically interacts with H3.1 raises interesting questions about the role of this interaction in terms of chromatin replication. Does the interaction indicate a specific role for POL2A in the insertion on chromatin of newly synthesized, unmodified H3.1 proteins over parental histones? Mono-, di-, and tri-methylation at H3K27 and H3K36 do not appear to have a major impact on the binding affinity of POL2A for H3.1 [39], arguing that modified (i.e., parental) histones may be a likely substrate for POL2A. If parental histones are inherited via POL2A, does it mean that parental H3.1-H4 dimers/tetramers are preferentially selected over parental H3.3-H4 during chromatin replication? Such a mechanism would have important implications for the mitotic inheritance of H3.3-enriched euchromatic states. A derived question would then be whether other DNA polymerase subunits or associated complexes can specifically transfer parental H3.1-H4 to the lagging strand during replication? If not, a unidirectional transfer of parental H3.1-H4 to the leading strand mediated by POL2A may be possible in some circumstances, and it could have functional ramifications for asymmetrical cell division [45]. Clearly, the discovery of the H3.1-POL2A interaction should lead to many interesting findings in the near future.

A substantial amount of work in plants has demonstrated the critical roles played by DNA polymerases in the mitotic inheritance of epigenetic states. Changes in active and repressive histone marks in hypomorphic mutants of plant DNA polymerases were shown to affect

developmental genes and the maintenance of heterochromatin structure and silencing, with these effects proposed to be either direct (e.g., disruption of the physical interaction between DNA polymerase epsilon and PRC2) or indirect (e.g., loss of epigenetic information due to replication stress) [46–52]. Evidence for these roles in mediating epigenetic inheritance was obtained mainly from phenotypic characterization and genomic profiling of transcriptomic and epigenomic states in DNA polymerase mutants. It will be important to revisit these findings and apply new molecular and sequencing tools to better understand the mechanisms by which the DNA replication machinery affects chromatin replication in plant model systems.

Conclusions and perspectives

The discovery that H3.1 can specifically interact with POL2A and TSK strongly suggests that many other proteins may preferentially bind the replication-dependent H3 variant, with consequences for chromatin replication and all other DNA-localized activities (e.g., DNA repair) that must be coordinated during S phase of the cell cycle. Further advances in this field will require the identification of these H3.1-binding proteins, and their functional characterization. Similarly, other histone variants in the H1, H2A, and H2B families (a single H4 variant is present in Arabidopsis [6]) may play specific roles during replication, which could be revealed by identifying proteins that can discriminate between closely-related variants in these families. Biochemical screens involving binding and modification assays are likely to be the fastest way to identify new cellular activities that rely on distinguishing minor sequence differences between histone variants.

Plants have made major contributions to the study of mitotic inheritance of epigenetic states, principally by being great model systems for deploying genetic and epigenomic tools. It will be important to keep expanding on what plant systems can do to remain important models in this field. For example, the use and optimization of plant cell lines could provide similar advantages to mammalian cells in working out the molecular roles of proteins involved in chromatin replication, while also revealing plant-specific functional variation in these mechanisms (e.g., the plant-specific role played by H3.1F41 in mediating spatial localization of H3.1 [53]). As replication-dependent H3 variants are present in all multicellular eukaryotes, advances in this field of research will continue to rely on the use of different biological systems. This is exemplified by the recent discovery that H3.1 play a role in mediating replication timing in mammals [54,55].

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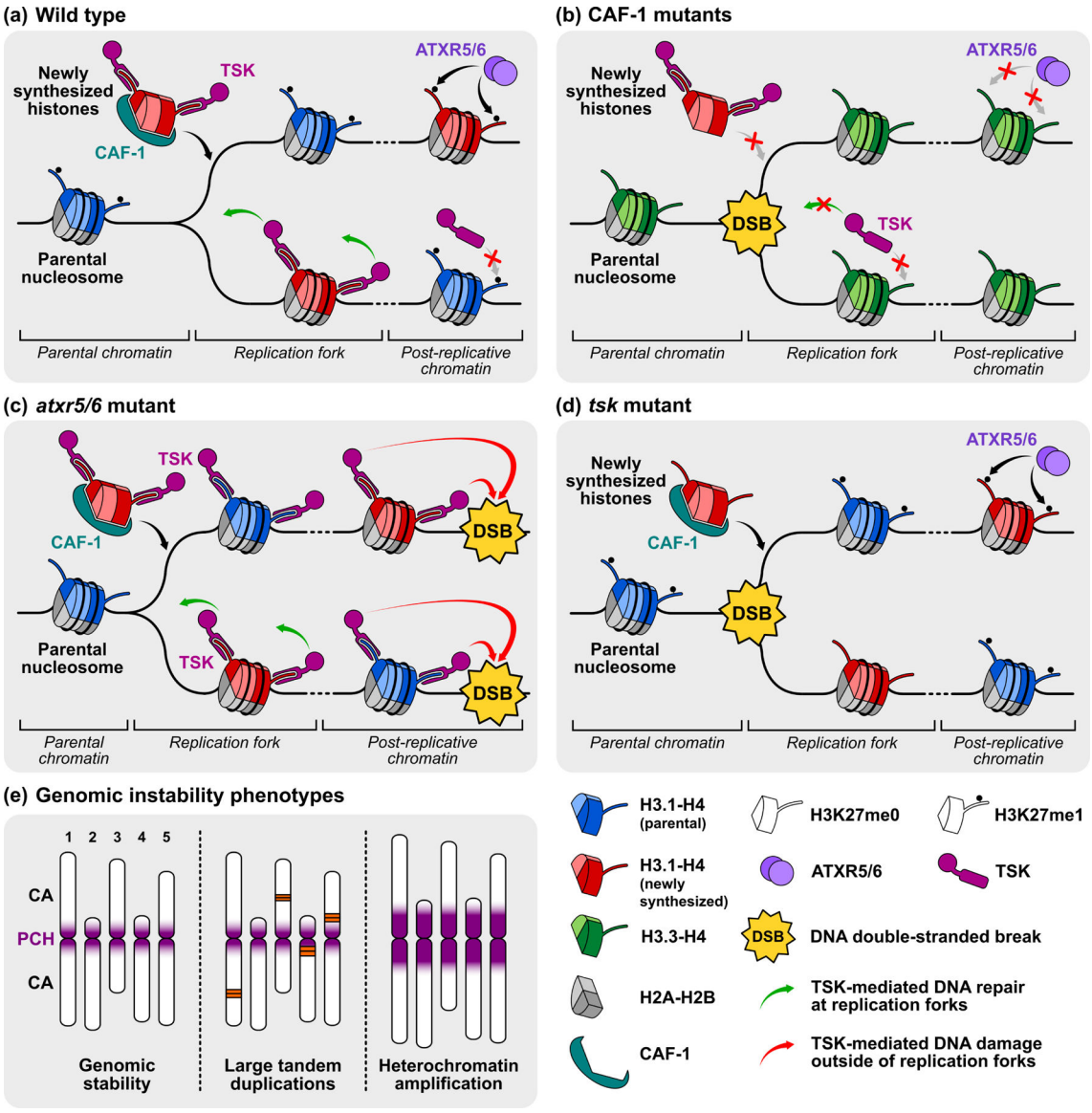

Figure 1. Interplay between H3.1 and genome stability in different genetic contexts.

(a-d) Chromatin replication in heterochromatin is represented, where parental nucleosomes are mainly composed of the H3.1 variant, except in the CAF-1 mutant. **(a)** In a wild-type plant, newly synthesized H3.1-H4 tetramers are loaded by the CAF-1 complex during replication. TSK binds H3.1K27me0 and resolves broken or stalled replication forks, ensuring genomic integrity. Post-replicative maturation of chromatin involves mono-methylation of H3.1K27 by ATXR5/6, which prevents ectopic binding of TSK. **(b)** In CAF-1 mutants, predominance of the H3.3 variant in chromatin prevents TSK activity, resulting in DNA damage at replication forks. **(c)** In the atxr5/6 mutant, absence of H3.1K27me1 causes aberrant binding of TSK and genomic instability outside of replication forks. **(d)** In a tsk context, H3.1 is loaded and mono-methylated correctly, but TSK is not available to resolve stalled or broken replication forks. **(e)** Schematic depiction of known H3.1-dependent structural phenotypes in the Arabidopsis genome: genomic stability (wild-

type), large tandem duplications (CAF-1 mutants), heterochromatin amplification (atxr5/6 mutants). PCH: pericentromeric heterochromatin; CA: chromosome arm.

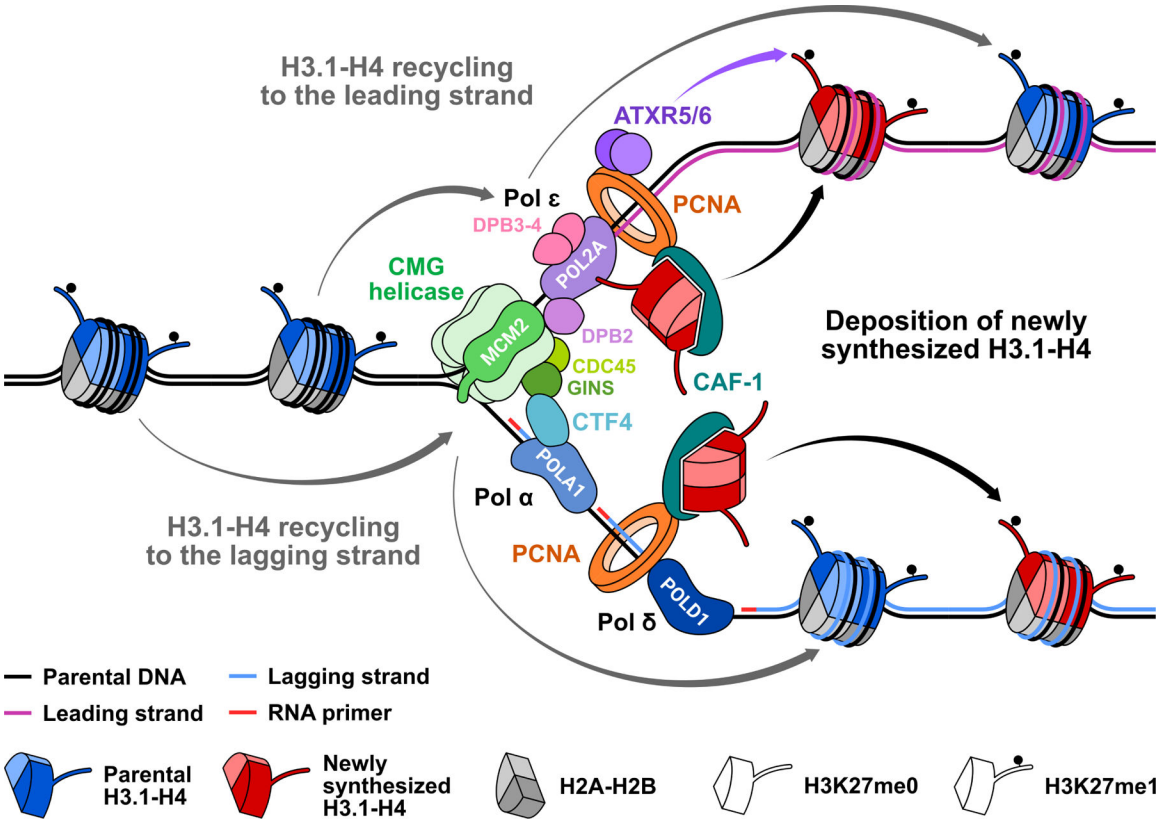

Figure 2. Interplay between H3.1 and DNA polymerases during replication.

In constitutive heterochromatin, parental nucleosomes are enriched in H3.1 mono-methylated at K27. At the replication fork, the CMG helicase, which includes MCM2–7, CDC45, and GINS, unwinds parental double stranded DNA. DNA polymerase epsilon (Pol ε) then synthesizes the leading strand, while DNA polymerases alpha (Pol α) and delta (Pol δ) synthesize the lagging strand, with their respective catalytic subunits POL2A, POLA1, and POLD1. The CAF-1 complex interacts with the PCNA clamp to load newly synthesized H3.1-H4 tetramers on both strands of the fork, after which ATXR5/6 deposit H3K27me1. Parental H3-H4 are recycled symmetrically to the leading and lagging strands. POL2A can bind H3.1 in its unmethylated (i.e., newly synthesized) and mono-methylated (i.e., parental) forms with potential implications for epigenetic inheritance. DPB3–4 are the plant homologs of mammalian POLE3–4, two non-catalytic subunits of Pol ε that were shown mediate recycling of parental H3-H4 to the leading strand. The MCM2-CTF4-Pol α axis is required for recycling of parental H3-H4 to the lagging strand in mammals, and may accomplish similar functions in plants.