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► **To cite this version:**

Ekaterina Boyarchuk, Rocío Montes de Oca, Geneviève Almouzni. Cell cycle dynamics of histone variants at the centromere, a model for chromosomal landmarks.. *Current Opinion in Cell Biology*, 2011, 23 (3), pp.266-76. <10.1016/j.ceb.2011.03.006>. <hal-00742742>

HAL Id: hal-00742742

<https://hal.science/hal-00742742v1>

Submitted on 17 Oct 2012

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Cell cycle dynamics of histone variants at the centromere, a model for chromosomal landmarks

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Running title: histone variants at the centromere

ABSTRACT

Classical heterochromatin chromosomal landmarks, such as centromeres and telomeres, are characterized by specific chromatin signatures. Among these, the incorporation of histone variants has recently emerged as an important feature. Using the centromere as a paradigm, we consider the role of histone variant dynamics in locus-specific chromatin organization. We describe the distinct location and dynamics of CenH3, H3.3, and H2AZ at the centromere during the cell cycle. This leads us to present the current view concerning modes of incorporation at this chromosomal landmark. Finally, we highlight the importance of histone variants in the crosstalk between the centric and pericentric domains for maintaining centromere identity.

INTRODUCTION

The classical chromosomal landmarks, centromeres and telomeres, identified cytologically on metaphase chromosomes are maintained as individual entities during interphase. Condensed throughout the cell cycle, they provide clear illustrations of the original definition of heterochromatin [1]. To date, additional molecular marks distinguish heterochromatin from euchromatin, including DNA methylation, non-coding RNAs, heterochromatin-associated proteins, and a combination of specific histone post-translational modifications (PTMs) imposed on particular histone variants. Recent data sparked attention to histone variants to understand their possible contribution in the identity of defined chromosome landmarks.

The two types of histone variants, canonical and replacement differ in their primary sequence, their expression during the cell cycle, their genome wide distribution and their mode of deposition [2] (Table 1). Canonical histones show a peak of expression during S phase and provide the main histone supply during DNA replication. They are mainly deposited in a DNA Synthesis-Coupled manner (DSC). In contrast, replacement variants are expressed independently of S phase, and are usually incorporated in a DNA Synthesis-Independent (DSI) manner. Challenging the notion that nucleosomes represent fairly stable biochemical entities, recent analyses with new technologies *in vivo* have shown high H3 turnover at defined transcribed and untranscribed regions, not only during genomic replication but also outside of S phase [3]. Therefore, nucleosome dynamics outside replication, when replacement variants are readily available should be considered. For a given variant, parameters affecting both *in vivo* nucleosome turnover (disassembly and reassembly events per unit of time) and local enrichment at particular loci are: (i) histone availability, (ii) presence of a functional deposition machinery, (iii) chromatin "receptivity" and (iv) stable maintenance. Additionally, the active recognition of a variant placement site, along with preventing its spreading to unwanted loci will favor locus-specific histone incorporation. Ultimately, the combination of all these aspects will enable the maintenance (or change) of a particular chromatin composition throughout multiple cell divisions.

In this review, we focus on the centromere as a paradigm for a locus marked by a specific H3 variant. We concentrate on the centromeric histone variant H3 (CenH3), H3.3, and H2AZ, and discuss recent advances concerning their incorporation and maintenance during the cell cycle.

MAIN TEXT

Centric and pericentric chromatin; a marking with histone variants

Centromeres, the chromosomal elements responsible for correct chromosome segregation during cell division, show a common chromatin organization [4]. Except in *S. cerevisiae*, where they are defined by a DNA sequence, in most eukaryotes centromeres assemble at repetitive sequences, yet these sequences are neither necessary nor sufficient for centromere formation. Thus,

centromere identity is considered of epigenetic nature, relying largely on chromatin features [4]. Within centromeres there are two chromatin domains: the centric chromatin, which serves as the site of kinetochore formation and is marked by the incorporation of a specific histone H3 variant, CenH3, and the adjacent pericentric heterochromatin (pHC), which lacks CenH3. The pericentric heterochromatin with highly methylated DNA, is enriched in hypoacetylated histones, H3K9me3, H3K9me2, H4K20me3, and accumulates Heterochromatin Protein 1 (HP1) [5,6]. In addition to the H3 variant CenH3, the presence of canonical H3, H3.3, and several H2A variants including H2AZ at centromeres (Table 1) prompts to evaluate our current knowledge concerning localization, timing, and mechanisms of deposition of these variants, to better understand how centromere organization is established and maintained throughout multiple cell divisions.

CenH3 at centric chromatin, its deposition and dynamics during the cell cycle

CenH3 (CENP-A in humans), first identified in sera from CREST patients, specifically localizes to centromeres. This variant, among the six H3 replacement variants (Table 1), is the most divergent with ~50-60% identity to canonical H3s at the histone fold domain and a unique N-terminal tail [4,7,8]. In different species CenH3s further diverge, yet all CenH3s share the ability to form nucleosome-like particles and organize centromeric chromatin to ensure proper centromere function. Importantly, maintenance of centromere identity largely depends on determinants involved on CenH3 deposition and stability. Depending on the specie, CenH3 deposition at centromeres occurs at different times [4]: in *S. cerevisiae*, all pre-existing CenH3^{Cse4} exchanges for new CenH3^{Cse4} during DNA replication, which once assembled at centromeres remains stable during most of the cell cycle. In *S. pombe*, major incorporation occurs at early S phase when CenH3^{Cnp1} expression levels peak (Figure 1), followed by further deposition during G2 phase, a potential salvage/fidelity pathway. In contrast, in all other organisms studied so far, CenH3 deposition at centromeres occurs outside of S phase, with variation in the exact timing of events. In *Drosophila*, CenH3^{CID} deposition occurs in early anaphase and mostly during G2 in plants. In humans, while CenH3^{CENP-A} expression peaks in late G2, new incorporation at centromeres occurs later during telophase and early G1 (Figure 1), when the overall CenH3^{CENP-A} is highly dynamic [9,10]. This replication of centromeric DNA during S phase without CenH3^{CENP-A} loading leads to the dilution of parental CenH3^{CENP-A} in half, with centromeric CenH3^{CENP-A} equally distributed to the daughter centromeres after each cell division [10]. Three non-mutually exclusive scenarios illustrate what occurs after DNA replication: (i) formation of hemisomes (documented in flies and humans [11,12]), (ii) formation of nucleosome gaps, or (iii) nucleosome gap-filling by a placeholder, either canonical H3 or H3.3, to be later replaced by CenH3 during the next cell cycle. While the actual nature and fate of centromeric particles during the cell cycle is still under debate, reaching a consensus on how to study nucleosome composition will be important, along with developing new technologies with enough resolution to test these scenarios.

Incorporation of CenH3 at centromeres, a complex and regulated process

Priming/licensing, deposition, and maintenance are three major events in CenH3 incorporation, which will be discussed here only in mammals. During priming, specific factors including the hMis18 complex (hMis18 α , hMis18 β , M18BP1 [Mis18-binding protein 1]/HsKNL2) and the histone chaperones RbAp46/48 are recruited to centromeres [13,14]. Additionally, changes in the acetylation status of centromeric histones are thought to make chromatin more receptive to CenH3 [13,14]. CenH3^{CENP-A} deposition during G1 uses only newly synthesized CenH3^{CENP-A} molecules [9,10] (Figure 1B). A critical chaperone required for CenH3 deposition *in vitro* and *in vivo* is the Holliday Junction-Recognizing Protein (HJURP) [15-17], which is also required for CenH3^{CENP-A} stability and impacts on proper chromosome segregation [17]. Moreover, pre-nucleosomal CenH3^{CENP-A} complexes also contain histone H4 and histone chaperones with broad histone specificity, that are also necessary for proper CenH3^{CENP-A} deposition [15,17] (Figure 2). Additional factors essential for CenH3 deposition are listed in Table 2. After deposition, “stabilizing/retaining” CenH3 only at centromeres (avoiding or removing spurious incorporations) is crucial for maintaining centromere identity and genomic stability. As candidate maintenance factors, the two subunits of the ATP-dependent nucleosome remodeling and spacing factor (RSF) complex, Rsf-1 and SNF2h, along with MgcRacGAP a GAP of the Rho family of small GTPases are particularly relevant [18,19] (Figure 1B; Table 2). The RSF complex, important for proper mitotic progression *in vivo*, can also reconstitute and space CenH3^{CENP-A} nucleosomes *in vitro* [19]. Meanwhile, MgcRacGAP together with the GEF ECT2 and their cognate small GTPase Cdc42 (or Rac) maintain CenH3^{CENP-A} at centromeres via a GTPase switch [18], and might mark newly incorporated CenH3^{CENP-A}. Finally, how priming, deposition, and maintenance are regulated, and which primary cue at centromeric chromatin triggers these events to ensure a regulated CenH3 dynamic cycle represent challenges for future study.

Canonical H3 and H3.3 at centromeres

The current view of centromeric structure organization based on chromatin fiber analysis from humans, mouse, and flies, proposes that within centric DNA, CenH3 subdomains are interspersed with histone H3 subdomains [4]. These centromeric H3 nucleosomes possess marks that differ from those at the flanking pericentric heterochromatin and euchromatin, and contain a specific combination of eu- and heterochromatic PTMs (detailed in Table 1). The presence of these marks at centric chromatin, especially H3K4me2, is important for HJURP recruitment to the centromere and for maintenance of CenH3 throughout multiple cell divisions [20]. Whether centromeric H3 nucleosomes contain canonical H3, H3.3, or a combination of both variants remains to be clarified.

The presence of H3.3 at pericentric heterochromatin is intriguing (Figure 2; Table 1), whether it localizes to the centric domain remains unclear. In mice, H3.3 becomes enriched at paternal chromosomes and this, along with methylation of H3.3K27 plays a critical role during *de*

novo heterochromatin formation at pericentric regions [21]. In human somatic cells, the pericentromeric but not the euchromatic H3.3 pool is phosphorylated in mitosis at serine 31 (H3.3S31p), a residue present only in H3.3 [22]. This mitotic phosphorylation also occurs in centromeric and telomeric heterochromatin in mouse embryonic stem cells (mESCs) [23]. However in these cells, satellite sequences, compared to other repeat sequences, show no particular enrichment in H3.3; accumulation only became evident upon differentiation [23-25]. Given the recently described importance of these transcripts during early development in mice [26], the presence of H3.3 at centromeres is perhaps a consequence of transcribing DNA satellite repeats. However, considering the lower stability reported for H3.3-containing nucleosomes [27,28], their presence within pericentric heterochromatin may promote the transcription of satellite repeats. Finally, H3.3 enrichment may simply reflect chromatin dynamics outside S phase, possibly occurring during reorganization of pHC and/or incorporation of CenH3.

Incorporation of H3.3 at pericentromeres

Deposition of H3.3 involves distinct histone chaperones [2] (Figure 2). The Histone Regulator A (HIRA) is part of a complex critical for DSI deposition of H3.3 *in vitro* [29]. HIRA is required for the global deposition of H3.3 during sperm remodeling in flies, and possibly in mice. In mESCs H3.3 enrichment at promoters and active gene bodies depends on HIRA [24] (Figure 2). An additional H3.3 associated complex contains the Death Domain-Associated (DAXX) and Alpha-Thalassemia/mental Retardation X-linked syndrome (ATRX) proteins. In this complex, DAXX serves as the H3.3 chaperone and requires ATRX for the enrichment at specific landmarks such as telomeres and pHC [24,25,30]. Interestingly, ATRX preferentially binds G-rich repetitive elements, predicted or known to form G-quadruplex structures, which include telomeric and mouse pericentric satellite repeats [31]. Importantly, loss of ATRX in human cells causes chromosome cohesion defects that lead to chromosome missegregation [32], and mutations in *Drosophila* ATRX homologues affect heterochromatin-mediated silencing [33]. To which extent these phenotypes are related to H3.3 loading at heterochromatic regions, or if they relate to other ATRX functions should stimulate future work. Several hypotheses can explain H3.3 maintenance at heterochromatic loci. First, the exchange of H3.3 during transcription of pericentromeric repeats, second, less removal from chromatin when compared to other loci in the genome, potentially due to the relatively compact nature of pHC. Third and non-exclusively, H3.3 could accumulate at centromeres by an active loading mechanism, similar to the one involved in loading newly synthesized H3.3 at telomeric heterochromatin during S phase in mESCs [34]. Coincidentally, while ATRX is constitutively found at pHC throughout the cell cycle, DAXX recruitment to these loci occurs during mid-late S phase, time of their replication [35]. Still, whether this S phase deposition of H3.3 is coupled to DNA synthesis or occurs shortly after fork passage in a DSI manner needs to be determined. Additional H3.3 deposition should also be considered at the time of centromere reorganization during mitosis and early G1 (Figure 1).

H2A variants at centromeres

In mammals, there are five H2A variants at centromeres (Table 1). Among them, H2AZ shows a direct role in centromere organization and function. Intriguingly, H2AZ enrichment at centromeres varies between species. In *S. cerevisiae*, centromeric nucleosomes are mostly devoid of H2AZ [36], while in *S. pombe* this remains controversial. Some reports suggest that H2AZ is part of the pHC, yet relatively depleted from centric chromatin [37], while others did not detect H2AZ at any position within the centromere [38,39]. Besides possible technical differences (ChIP vs ChIP-Seq), a relatively low enrichment of H2AZ at yeast centromeres may have caused high variability in these analyses. In mammals, H2AZ is present in both pericentric and centric chromatin [40]. Interestingly, centromeric H2AZ is also maintained in both the inactive X-chromosome, generally depleted of H2AZ [40], and in mature human sperm, where less than 10% of the histones are retained [41,42]; supporting a significant role for H2AZ at the centromere and a transgenerational inheritance. In *S. pombe*, loss of H2AZ^{Pht1} causes defects in silencing at centromeres [39]. In mouse cells, depletion of H2AZ results in changes in pHC structure during both interphase and mitosis, and formation of inter-chromosomal bridges containing major satellite repeats [40]. Thus, it is tempting to speculate that the major phenotype following global H2AZ depletion consisting in chromosome segregation defects [37,40], relates directly to H2AZ perturbations in pHC. Alternatively, it was proposed that in *S. pombe* the genomic instability caused by H2AZ^{Pht1} depletion was due to H2AZ's function in RNA processing [38].

H2AZ incorporation

The incorporation of H2AZ onto chromatin requires coordination between histone chaperones and chromatin remodelers. In all organisms tested, unincorporated H2AZ-H2B dimers are in complex with the nucleosome assembly protein 1 (Nap1), a known chaperone for H2A-H2B, which facilitates H2AZ exchange *in vitro* [38,43]. The function of an additional H2AZ^{Htz1} specific chaperone, identified in budding yeast, Chz1, partially overlaps with Nap1, and other chaperones can substitute for both Nap1 and Chz1 *in vivo* [43]. In *S. cerevisiae* and *S. pombe*, the exchange of H2A for H2AZ on chromatin is carried out by the chromatin remodeling complex SWR1 (SWR1-C) [38,44]. The catalytic subunit of SWR1-C, Swr1, has an ATPase/helicase domain homologous to that of Swi2/Snf2 remodelers and is essential for genome-wide incorporation of H2AZ [44]. In *S. pombe*, the absence of Swr1 results in the global loss of H2AZ^{Pht1} throughout the genome with a relative accumulation of H2AZ^{Pht1} at core centromeric and subtelomeric regions [45]. These data suggest the existence of other loading complex(es) that can deposit H2AZ^{Pht1} at these heterochromatic loci. In *S. cerevisiae*, the genome-wide distribution of H2AZ is further tuned by the eviction of mislocalized H2AZ by another member of the Swi2/Snf2 remodelers, the INO80 complex, which can replace nucleosomal H2AZ-H2B with free H2A-H2B dimers *in vivo* and *in vitro* [46]. This *in vitro* activity of INO80 towards H2AZ-H2B remains controversial [46,47]; the

discrepancy is likely due to different experimental conditions, as INO80 activity seems to require relatively high concentration of substrate [46]. In higher order eukaryotes, members of the SWI2/SNF2 family of remodelers are also involved in H2AZ exchange [44]. For example, the *Drosophila* TIP60 complex, which contains the homologue of Swr1 (Domino/p400) can catalyze the exchange of H2AZ^{H2Av}. In human cells, TIP60 and SRCAP (SWI2/SNF2-related CBP activator protein) complexes are required for incorporation of H2AZ at defined promoters *in vivo* (Figure 2). Interestingly, two components of SRCAP, the ATP-dependent helicases TIP48 and TIP49 (also known as TIP49a/b), can exchange H2AZ-H2B dimers and show a preference for H2AZ over H2A or H2AX *in vitro* [48]. Notably, TIP48 and TIP49 are components of several other chromatin complexes [49]. Thus, future studies should evaluate how different chromatin remodelers together with the network of chromatin chaperones mediate H2AZ/H2A exchange, and help better explain H2AZ accumulation at distinct chromosomal landmarks.

Crosstalk between centric and pericentric domains

Propagation of centromere integrity involves interactions between centric and pericentric chromatin. In *S. pombe*, pHC is essential for *de novo* establishment of CenH3 chromatin [50,51], which might also serve as a boundary for limiting CenH3 spreading as illustrated in *Drosophila*, where pHC facilitates proper CenH3^{CID} localization [52]. Similarly, in artificial human chromosomes, some CenH3 spreading occurs due to the transient overexpression of CenH3^{Cenp-A}. In this context, CenH3^{Cenp-A} displaces neighboring H3K9me2, but not H3K9me3 nucleosomes, a hallmark of pHC [53]. Additionally, histone variants might serve as barriers between centric and pericentric chromatin. For instance, ectopic enrichment of H2AZ^{Pht1} at centric chromatin results in an increase of canonical H3 occupancy at this region [45], and this coincides with decreased CenH3^{Cnp1} [54], suggesting that H2AZ^{Pht1} might antagonize CenH3 incorporation at the domain. Thus, pHC both promotes and antagonizes formation of the centric domain. Intriguingly, in yeast and mammals, the initiation of CenH3 deposition coincides with the partial disruption of pHC organization (Figure 1C). In *S. pombe*, Swi6 (the HP1 homologue) is partially released from centromeres at the beginning of mitosis due to a phospho-methyl switch involving phosphorylation of H3S10 and partial loss of H3K9me2 (Figure 1C; left). Full restoration of pHC organization occurs in late S phase, after the loading of CenH3 (early S phase) [5]. In mammals, there is also substantial HP1 dissociation from pHC during late G2/early M phase (Figure 1C; right), and HP1 re-accumulates at this domain during G1 [55]. This restoration likely occurs after centromeric chromatin “priming” and initiation of CenH3 deposition [10,14]. How these two domains are disrupted and restored in a coordinated and timely manner will be an important question to address. Notably, this partial disruption of pHC organization during mitosis can provide a window of opportunity for priming centric chromatin, the initial targeting of the CenH3 pre-deposition complex, and perhaps the incorporation of CenH3. These changes in chromatin organization might also facilitate the eviction and replacement by CenH3 of other H3 variants used as placeholders during the dilution of CenH3 in S phase. Once

CenH3 deposition is initiated, restoration of pHC organization would prevent promiscuous spreading of CenH3 and facilitate the retention of this variant at the domain. Reciprocally, spreading of heterochromatin marks to centric chromatin should also be prevented. In human artificial chromosomes, targeting of a transcriptional repressor to the centromere results in the strong accumulation of H3K9me3 and the binding of HP1 α at the centric domain, which coincides with the gradual loss of CenH3 from centromeres and kinetochore inactivation [56]. How centric and pericentric domain boundaries are mechanistically established is still a puzzle. One possibility includes a rapid histone turnover at the boundary, which might serve to separate chromatin domains [57]. Indeed, in *S. cerevisiae* nucleosomes containing hyperacetylated H2AZ^{Htz1}, which exhibit a high turnover rate, are necessary to prevent the spreading of heterochromatin factors from the adjacent subtelomeric regions [58,59]. In mammals, nucleosomes containing both H2AZ and H3.3 are unstable *in vivo* [27]. Thus, searching for the presence of double-variant-containing particles at centromeres as a means to form a barrier between centric and pericentric chromatin represents an attractive hypothesis to explore.

CONCLUSIONS

We have discussed how histone variants contribute to centromere specification, organization, maintenance, and function. The precise and regulated incorporation of CenH3 at centromeres presents a major challenge to preserve centromere identity. Whether the incorporation of other histone variants including H3.3 and H2AZ at centromeres is as tightly regulated remains to be tested. The existence of an H3.3 complex (ATRX-DAXX) important for H3.3 enrichment at heterochromatic regions is highly suggestive of a regulated mechanism. Although CenH3 is a key determinant for centromere identity it is not the only factor to be considered. Incorporation of other histone variants carrying specific posttranslational modifications, association with other proteins (e.g., the constitutive centromere-associated network, CCAN; Table 2), non-coding RNAs, and a particular 3D organization of the domain, are important candidates to be further evaluated. Furthermore, the current view suggests that the centromere 3D organization may act as a feedback loop that controls centromere identity and propagation [4,10]. In particular, Condensins, which form protein complexes that contribute to higher-order chromatin organization, are important for deposition and retention of CenH3 at centromeres in several organisms [60-62]. To enrich our understanding of centromere dynamics we should also account for changes that occur during both the cell cycle and development, when major rearrangements take place and cells exhibit different proliferation abilities. Finally, beyond thinking of this structure as an individual entity, considering the “local neighborhood” and the crosstalk between domains within the nuclear space should promote new research avenues.

ACKNOWLEDGEMENTS

We apologize to colleagues whose work could not be cited due to space constraints. We thank D. Ray-Gallet, A. Cook and Z. Gurard-Levin for discussions and critical reading. This work was supported by la Ligue Nationale contre le Cancer (Equipe labellisée Ligue 2010 to G. A and postdoctoral fellowship to R. M), ANR "ECenS" ANR-09-BLAN-0257-01 and ERC Advanced Grant 2009-AdG_20090506 "Eccentric".

GLOSSARY

Location/localization: presence of a given histone variant at a defined site.

Deposition/incorporation: loading of a given histone variant potentially involving specific histone chaperones, remodelers and/or modifiers.

Maintenance: an active process to keep a mark during multiple cellular divisions.

FIGURE LEGENDS

Figure 1. Comparison of the propagation of centric and pericentric chromatin during the cell cycle in *S. pombe* and mammals. A) Schematic of centromere composition and organization. Two domains are distinguished, a central domain, centric chromatin and the flanking pericentric heterochromatin (pHC). Centromere DNA sequence and size varies between species. In the *S. pombe* centromere, the central core region is flanked by innermost (*imr*) and pericentric outer repeats, while the *M. musculus* centromeres contain tandem repeats of centric minor (~600 kb) and pericentric major (~6 Mb) satellite DNA. Both domains show characteristic chromatin marks including the histone variant CenH3 (*SpCnp1*, *MmCENP-A*) in the centric domain, and the presence of heterochromatin protein 1 (*SpSwi6* in *S. pombe*, HP1 in mammals) in the pericentric regions. We show factors important for the propagation of the centric and pericentric chromatin (purple and green, respectively). **B)** CenH3 propagation during the cell cycle involves: 1) priming/licensing as identified in humans, which occurs during anaphase/telophase to early G1 and depends on the hMis18 complex and the chaperones RbAp46/48; 2) deposition, which occurs during S and G2 phases in *S. pombe*, and from telophase to early G1 phase and involves the CenH3 specific chaperone HJURP in mammals; and 3) CenH3 maintenance during late G1, as described in mammals, and depends on the RSF complex and the MgcRacGAP small GTPase. Importantly, since CenH3 deposition is not coupled to centromeric DNA replication in mammalian cells, the CenH3^{CENP-A} protein pool is diluted in half after S phase. This dilution leads to three potential scenarios: formation of hemisomes, nucleosome gaps, or the use of other histone variants as placeholders until the end of mitosis, when newly synthesized CenH3^{CENP-A} will be deposited and presumably will replace these variants. **C)** Similarly to CenH3, pericentric heterochromatin is also propagated in a regulated manner as shown in the overlaid schematic. HP1 (*SpSwi6*) partially dissociates from pHC at the beginning of mitosis due to a phospho-methyl switch (H3S10p vs H3K9me2). In *S. pombe*, pHC organization is restored in S phase after centromeric DNA replication resulting in further dilution of heterochromatic marks. This restoration involves RNAi-dependent and independent pathways that recruit histone-modifying enzymes to reconstitute the typical heterochromatic signatures of pHC: *SpSwi6*, H3K9me2, and hypoacetylated histones. In mammals, HP1 levels are restored at the beginning of G1 phase. Later, HP1 and the heterochromatic histone marks are maintained throughout replication. Fidelity of this process is ensured by the cooperation between the DNA replication machinery and multiple DNA and histone modifying complexes at the replication fork, including the histone methyltransferase Suv39h (*SpClr4*), the CAF-1 chaperone complex (p150 subunit, *Sppcf1*), and histone deacetylases (only known in *S. pombe*, *Clr3*, *Clr6*). Thus, full heterochromatic structure is restored after fork passage [6,63]. Replication-coupled maintenance of heterochromatin components also contributes to the restoration of pHC structure in yeast [6]. The priming of centric chromatin and the initiation of CenH3 deposition coincide with the temporal disruption of pHC organization in both organisms, which might facilitate CenH3 loading at centromeres.

Figure 2. Distribution of histone variants at mammalian centromeres. Deposition of CenH3, H3.3, and H2AZ at different genome locations in mammals. Schematic of an acrocentric chromosome depicting the chromosomal landmarks, telomeres and centromere. Two domains form the centromere, centric (purple) and pericentric (green), at which several histone variants are incorporated by specific chaperones as indicated. H3.3 localization at telomeres and pericentric heterochromatin is dependent on the ATRX/DAXX complex, while presence at active promoters depends on the HIRA complex [24,25]. Note that localization of H3.3 at telomeres is only documented in mESCs [23,24]. The presence of H2AZ at promoters requires the SRCAP complex and/or TIP60 [44]. How H2AZ locates at heterochromatic loci remains unknown. The deposition of CenH3^{CENP-A} at centric chromatin requires the CenH3-specific chaperone HJURP [15,17]. Other histone chaperones might aid in deposition and/or eviction of histone variants including the FACT complex, RbAp46/48, nucleophosmin 1 (NPM1), and the chromatin remodeling complex RSF, in which the Rsf-1 subunit serves as the chaperone [14,17,19,64].

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Table 1. Distribution and posttranslational modifications of eukaryotic histone variants

| Histone variant | | Conservation | Distribution | PTMs at mammalian centromeres |
|-----------------|--------------------------------------|--|--|---|
| Family | Variant/Species | | | |
| H3 | H3 (canonical) Ubiquitous | <i>Sc, Sp</i> : H3 ^a <i>Dm, Xt</i> : H3.2 <i>Mm, Hs</i> : H3.1 & H3.2 | Global | <i>Centric</i> : H3K4me2 ^[4] , H3K56me2/3, H3K9me2/3 ^[20] , Hypoacetylated ^[5] <i>Pericentric</i> : H3K9Me2/3, H3K27me ^[5] , H3T3p (mitosis) ^[65] , Hypoacetylated ^[5] |
| | H3.3 Metazoan | <i>Dm, Xt, Mm, Hs</i> : H3.3 | Promoters and active gene bodies, gene regulatory elements. <i>Mm</i> : telomeres, meiotic XY body. <i>Mm, Hs</i> : Centromeres . <i>Dm, Mm</i> : Paternal chromatin at fertilization ^[2] | <i>Centric</i> : ND <i>Pericentric</i> : H3.3S31p (mitosis) ^[22] , H3.3K27me (paternal pronucleus) ^[21] |
| | CenH3 Ubiquitous | <i>Sc</i> Cse4 <i>Sp</i> Cnp1 <i>Dm</i> CID <i>Xt, Mm, Hs</i> : CENP-A | Centromeres <i>Sc</i> : Regions with high histone turnover, tRNA genes ^[66,67] <i>Hs</i> : DNA breaks ^b ^[68] | <i>Centric</i> : CENP-A S7p (mitosis) ^[69] <i>Pericentric</i> : NA |
| | H3t Mammals ^c | <i>Mm, Hs</i> : H3t | ND (sperm) ^[7] Nucleolus (somatic cells) ^[70] | NA |
| | H3.X/Y Primates | <i>Hs</i> : H3.X & H3.Y | Euchromatin ^[71] | NA |
| | H3.5 Hominids | <i>Hs</i> : H3.5 | Testis-specific, euchromatin ^[72] | NA |
| H4 | H4 (canonical) Ubiquitous | H4 | Global | <i>Centric, Pericentric</i> : H4K20me3 Hypoacetylated ^[5] |
| H2A | H2A (canonical) Ubiquitous | H2A ^d | Global | <i>Centric</i> : H2AT120p (mitosis) ^[65] <i>Pericentric</i> : ND |
| | H2A.X Metazoan | <i>Dm</i> H2Av ^e <i>Xt, Mm, Hs</i> : H2A.X | Global | <i>Centric, Pericentric</i> : H2AXS139p exclusively at the periphery of the centromere ^[73] |
| | H2AZ Ubiquitous | <i>Sc</i> Htz1, <i>Sp</i> Pht1 <i>Dm</i> H2Av ^e <i>Xt</i> H2A.ZI <i>Mm, Hs</i> : H2AZ1 & 2 | Promoters and the body of active and inducible genes, gene regulatory elements ^[7] , nucleolus ^[70] . <i>Sc, Sp</i> : subtelomeric regions ^[38,58] . <i>Sp, Dm, Mm, Hs</i> : centromeres ^[7,37,40] <i>Mm</i> : meiotic XY body ^[74] | <i>Centric, Pericentric</i> : ND |
| | Macro-H2A 1,2 Amniotes | <i>Gg</i> : mH2A.1 & mH2A.2 <i>Mm, Hs</i> : mH2A.1-1, -2 & mH2A.2 | Inactive X-chromosome, promoters of imprinted genes, promoters of inducible developmental genes ^[7] , telomeres ^[75] , centromeres ^[76,77] , nucleolus ^[70] , meiotic XY body ^[77] | <i>Centric, Pericentric</i> : mH2A S137p excluded ^[78] |
| | H2AL1, L2 Rodent | <i>Mm</i> H2AL1 & H2AL2 | Centromeres (sperm) ^[7] | ND |
| | H2ABbd Mammals | <i>Mm, Hs</i> : H2ABbd | Euchromatin ^[7] | NA |
| H2B | H2B (canonical) Ubiquitous | H2B | Global | <i>Centric, pericentric</i> : H2BT119p (mitosis) ^[79] |

| | | | | |
|--|--------------------------------------|--------------------------------------|--|----|
| | TSH2B Mammals ^f | <i>Mm, Hs:</i> TSH2B | Global (sperm) ^[7] Telomeres (somatic cells) ^[75] | NA |
| | H2BFWT Mammals | <i>Ms: H2BL1</i> <i>Hs: H2BWT</i> | Telomeres (sperm) ^[7] | NA |

Footnotes:

NA, not applicable; ND, not determined; *Sc, S. cerevisiae*; *Sp, S. pombe*; *Dm, D. melanogaster*; *Xl, X. laevis*; *Gg, G. gallus*; *Mm, M. musculus*; *Hs, H. sapiens*

^a CenH3 and H3 are the only two H3 variants in yeast. In yeast the “canonical” H3 is related to the metazoan H3.3, and can be deposited onto chromatin in both DNA synthesis-coupled and -independent manners [2]

^b The transient accumulation of GFP-CENP-A at DNA breaks might be a result of CENP-A overexpression

^c H3t, originally reported as testis specific, was also found in a nucleolus proteome from HeLa cells [70].

Note that the tumoral origin of HeLa cells might have caused the expression of H3t in this somatic cell line.

^d H2A and H2AZ are the only two H2A variants in yeast. H2A is referred as canonical H2A; however, it functions similarly to vertebrate H2AX during DNA damage responses (e.g., it is phosphorylated at DNA damage sites)

^e H2Av is a hybrid between H2AX and H2AZ, it contains an SQ(E/D)F motif at its C-terminus and becomes phosphorylated in response to DNA damage [7]

^f TSH2B, originally reported as a testis specific variant, it was recently found in association with telomeric repeats in several human cell lines [75]

Table 2. Factors required for CenH3 incorporation at mammalian centromeres

| Function during CenH3 incorporation | Factor | Conservation | Properties | Centromeric localization during cell cycle | Refs |
|---|---|---|--|---|---------|
| Priming/licensing | RbAp46/48 | <i>Sp</i> Mis16 | General chaperone for H3-H4 and potentially CenH3 ^{CENP-A} . Might alter chromatin acetylation status | ND | [14,17] |
| Priming/licensing | Mis18 α/β | <i>Sp</i> Mis18 | Part of the hMis18 complex, which does not associate with CenH3 ^{CENP-A} <i>in vivo</i> | Late anaphase/ telophase \rightarrow early G1 | [14] |
| Priming/licensing | M18BP1/HsKNL2 | <i>Ce</i> KNL-2 | Part of the hMis18 complex, which does not associate with CenH3 ^{CENP-A} <i>in vivo</i> | Late anaphase/ telophase \rightarrow early G1 | [14] |
| Deposition <i>in vitro</i> & <i>in vivo</i> | HJURP | <i>Sc</i> Scm3 <i>Sp</i> Scm3 <i>Xl</i> HJURP | CenH3 ^{CENP-A} specific chaperone | Late telophase \rightarrow early G1 | [15-17] |
| Deposition | FACT complex (SSRP1, SPT16) | <i>Dm</i> Ssrp1 <i>Sp</i> Pob3 <i>Sc</i> Spt16 <i>Sp</i> Spt16 <i>Dm</i> Dre4 | General chaperone for H3-H4, H2A-H2B, and potentially for CenH3 ^{CENP-A} | Constitutive | [64] |
| Deposition | CHD1 | <i>Sp</i> Hrp1 | Chromatin remodeling factor | Constitutive | [64] |
| Deposition | Constitutive Centromere-associated network (CCAN) | CENP-O CENP-P CENP-Q CENP-R CENP-U/50/KLIP-1 CENP-L / (<i>Sp</i> SPAC4F10.12) CENP-M (<i>Sp</i> Mis17) CENP-N (<i>Sp</i> Mis15) CENP-S CENP-T CENP-H / (<i>Sp</i> Sim4) CENP-I / (<i>Sp</i> Mis6) CENP-K | Multiprotein complex required for centromere specification and kinetochore assembly | Constitutive | [8] |
| Maintenance | RSF complex (Rsf-1, SNF2h) | <i>Dm</i> Rsf-1, <i>Sc</i> lsw1 | ATP-dependent chromatin remodeling complex | Mid-G1 \rightarrow M | [19] |
| Maintenance | MgcRacGAP | | GAP for the Rho family of small GTPases. Associates with the nucleotide exchange factor (GEF) Ect2, and the small GTPases Cdc42 and Rac. Interacts with the licensing factor M18BP1/HsKNL2 | Only in late-G1 | [18] |

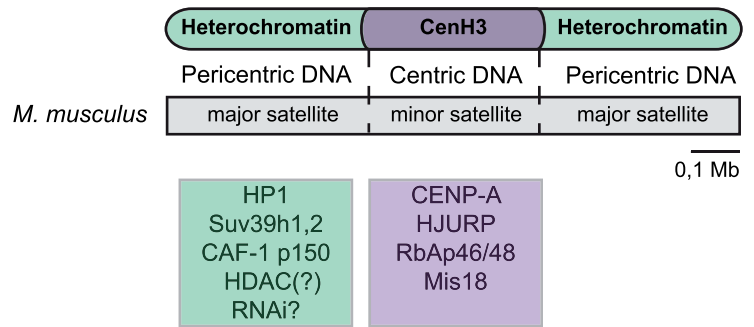
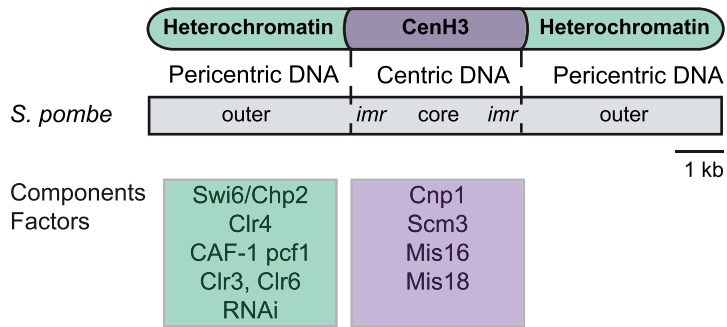
Footnotes:

Sc, *S. cerevisiae*; *Sp*, *S. pombe*; *Xl*, *X. laevis*; *Ce*, *C. elegans*; *Hs*, *H. sapiens*. ND, not determined

Figure 1

Figure 1.

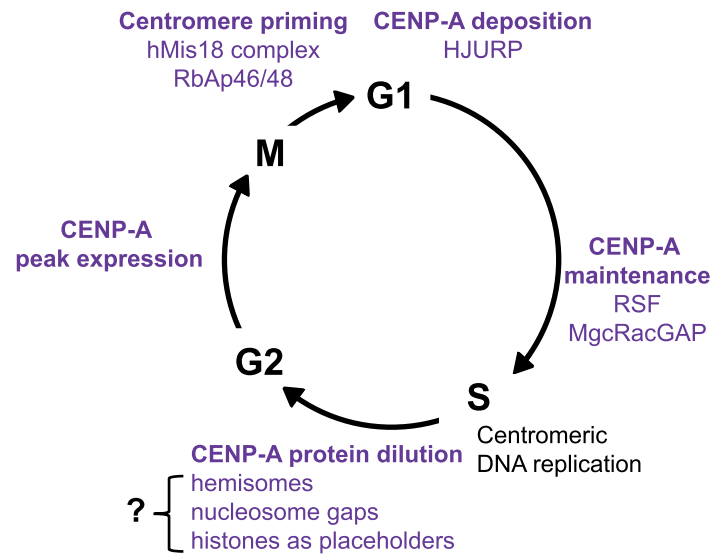
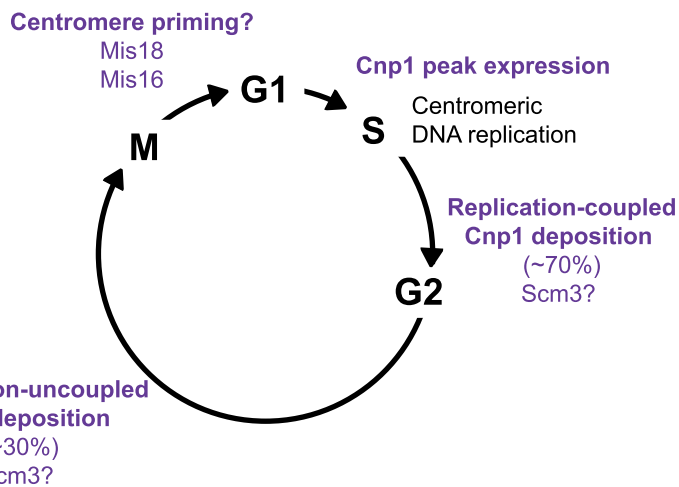
A)



B)

S. pombe

Mammals



C)

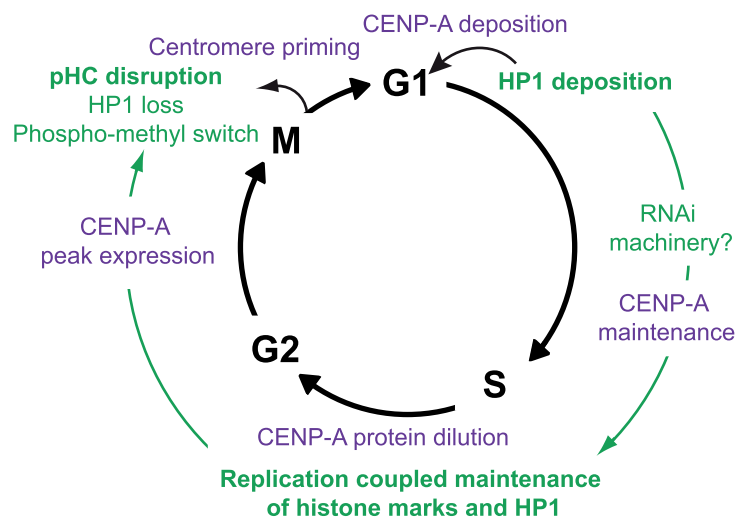
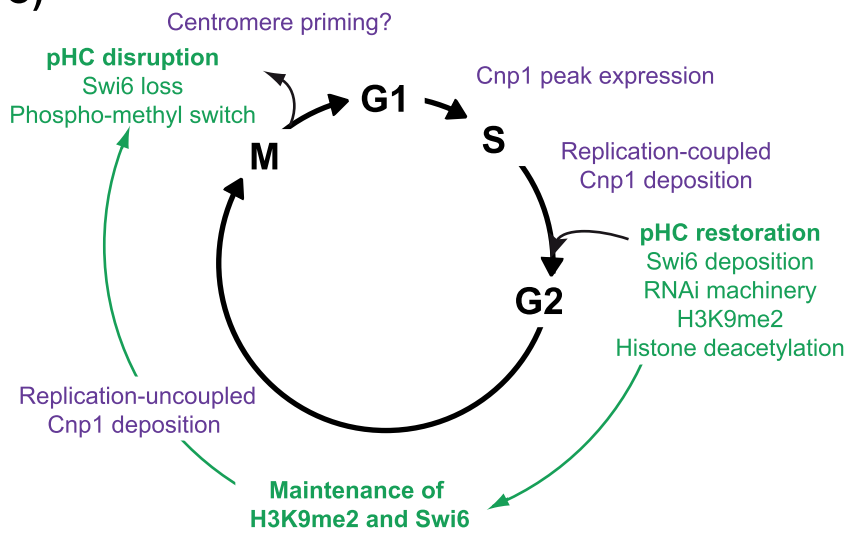


Figure 2

Figure 2.

