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

Ekaterina Boyarchuk, Rocío Montes de Oca, Geneviève Almouzni

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

Ekaterina Boyarchuk[# 1,2], Rocío Montes de Oca[# 1,2] and Geneviève Almouzni[1,2]

[#] these authors contributed equally to this manuscript

[1]Institut Curie, Centre de Recherche, Paris, F-75248 France

[2]CNRS, UMR218 - Laboratory of Nuclear dynamics and Genome plasticity, Paris, F-75248 France

[*]*Corresponding author:*

Geneviève Almouzni

Address: Institut Curie, Centre de Recherche, CNRS - UMR218, Laboratory of Nuclear dynamics and Genome plasticity, 26 rue d'Ulm, 75248 Paris Cedex 05, France

Phone: +33-1 56 24 67 01

Fax: +33-1 46 33 30 16

E-mail: genevieve.almouzni@curie.fr

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 (hMis18α, hMis18β, 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]. $CenH3^{CENP\text{-}A}$ deposition during G1 uses only newly synthesized $CenH3^{CENP\text{-}A}$ 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 $CenH3^{CENP\text{-}A}$ stability and impacts on proper chromosome segregation [17]. Moreover, pre-nucleosomal $CenH3^{CENP\text{-}A}$ complexes also contain histone H4 and histone chaperones with broad histone specificity, that are also necessary for proper $CenH3^{CENP\text{-}A}$ 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 $CenH3^{CENP\text{-}A}$ nucleosomes *in vitro* [19]. Meanwhile, MgcRacGAP together with the GEF ECT2 and their cognate small GTPase Cdc42 (or Rac) maintain $CenH3^{CENP\text{-}A}$ at centromeres via a GTPase switch [18], and might mark newly incorporated $CenH3^{CENP\text{-}A}$. 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 heterochoromatic 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 $H2AZ^{Pth1}$ 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 $CenH3^{Cenp\text{-}A}$. In this context, $CenH3^{Cenp\text{-}A}$ 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 $H2AZ^{Pth1}$ 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 HP1α 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 constrains. 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 (*Sp*Cnp1, *Mm*CENP-A) in the centric domain, and the presence of heterochromatin protein 1 (Swi6 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 $CenH3^{CENP\text{-}A}$ 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 $CenH3^{CENP\text{-}A}$ 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 (*Sp*Swi6) 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 restitute the typical heterochromatic signatures of pHC: Swi6, 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 (*Sp*Clr4), the CAF-1 chaperone complex (p150 subunit, *Sp*pcf1), 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 $CenH3^{CENP\text{-}A}$ 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].

REFERENCES AND RECOMMENDED READING

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

• of special interest

•• of outstanding interest

1. Heitz E: **Das heterochromatin der Moose**. *Jehrb Wiss Botanik* 1928, **69**:762-818.
2. Szenker E, Ray-Gallet D, Almouzni G: **The double face of the histone variant H3.3**. *Cell Res* 2011.
3. • Deal RB, Henikoff JG, Henikoff S: **Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones**. *Science* 2010, **328**:1161-1164.

The authors develop a new technique, CATCH-IT, to measure genome-wide histone H3 turnover. and find differential histone turnover across the genome.

4. Allshire RC, Karpen GH: **Epigenetic regulation of centromeric chromatin: old dogs, new tricks?** *Nat Rev Genet* 2008, **9**:923-937.
5. Probst AV, Dunleavy E, Almouzni G: **Epigenetic inheritance during the cell cycle**. *Nat Rev Mol Cell Biol* 2009, **10**:192-206.
6. Maison C, Quivy JP, Probst AV, Almouzni G: **Heterochromatin at Mouse Pericentromeres: A Model for De Novo Heterochromatin Formation and Duplication during Replication**. *Cold Spring Harbor Symposia on Quantitative Biology* 2011.
7. Talbert PB, Henikoff S: **Histone variants--ancient wrap artists of the epigenome**. *Nat Rev Mol Cell Biol* 2010, **11**:264-275.
8. Cheeseman IM, Desai A: **Molecular architecture of the kinetochore-microtubule interface**. *Nat Rev Mol Cell Biol* 2008, **9**:33-46.
9. Hemmerich P, Weidtkamp-Peters S, Hoischen C, Schmiedeberg L, Erliandri I, Diekmann S: **Dynamics of inner kinetochore assembly and maintenance in living cells**. *The Journal of Cell Biology* 2008, **180**:1101-1114.
10. Jansen LE, Black BE, Foltz DR, Cleveland DW: **Propagation of centromeric chromatin requires exit from mitosis**. *J Cell Biol* 2007, **176**:795-805.
11. Dalal Y, Wang H, Lindsay S, Henikoff S: **Tetrameric structure of centromeric nucleosomes in interphase Drosophila cells**. *PLoS Biol* 2007, **5**:e218.
12. • Dimitriadis EK, Weber C, Gill RK, Diekmann S, Dalal Y: **Tetrameric organization of vertebrate centromeric nucleosomes**. *Proc Natl Acad Sci U S A* 2010.

Using a combination of atomic force and immunoelectron microscopy, the authors revealed the presence of tetrameric CENP-A containing particles (hemisomes) during interphase in human cells.

13. Hayashi T, Fujita Y, Iwasaki O, Adachi Y, Takahashi K, Yanagida M: **Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres**. *Cell* 2004, **118**:715-729.
14. Fujita Y, Hayashi T, Kiyomitsu T, Toyoda Y, Kokubu A, Obuse C, Yanagida M: **Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1**. *Dev Cell* 2007, **12**:17-30.
15. •• Foltz DR, Jansen LET, Bailey AO, Yates Iii JR, Bassett EA, Wood S, Black BE, Cleveland DW: **Centromere-Specific Assembly of CENP-A Nucleosomes Is Mediated by HJURP**. *Cell* 2009, **137**:472-484.

This study in parallel with the work published by Foltz *et al*., 2009 (also cited) isolated pre-nucleosomal CENP-A complex components. From these complexes, they identified the Holliday Junction-Recognizing Protein (HJURP) as the chaperone required for $CenH3^{CENP-A}$ deposition. Additionally, they demonstrated that HJURP is required for CENP-A stability and for proper chromosome segregation.

16. •• Shuaib M, Ouararhni K, Dimitrov S, Hamiche A: **HJURP binds CENP-A via a highly conserved N-terminal domain and mediates its deposition at centromeres**. *Proceedings of the National Academy of Sciences* 2010, **107**:1349-1354.

The authors biochemically identified the direct binding sites between HJURP and CENP-A, the TLTY box and the CATD respectively. They also showed that HJURP facilitates the *in vitro* deposition of CENP-A-H4 tetrameres onto DNA.

17. •• Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D, Nakamura Y, Daigo Y, Nakatani Y, Almouzni-Pettinotti G: **HJURP Is a Cell-Cycle-Dependent Maintenance and Deposition Factor of CENP-A at Centromeres**. *Cell* 2009, **137**:485-497.
See the reference from Foltz *et al*., 2009.

18. •• Lagana A, Dorn JF, De Rop V, Ladouceur AM, Maddox AS, Maddox PS: **A small GTPase molecular switch regulates epigenetic centromere maintenance by stabilizing newly incorporated CENP-A**. *Nat Cell Biol* 2010, **12**:1186-1193.
Using a combination of methods including high-resolution time microscopy and fluorescently tagged proteins the authors identified a GAP from the Rho family of small GTPases, MgcRacGAP, as a key factor for CENP-A maintenance. This GAP only localizes briefly to centromeres, in late G1, and drives a GTPase switch that is essential for retaining newly-assembled CENP-A at centromeres.

19. •• Perpelescu M, Nozaki N, Obuse C, Yang H, Yoda K: **Active establishment of centromeric CENP-A chromatin by RSF complex**. *The Journal of Cell Biology* 2009, **185**:397-407.
The authors characterized the dynamics of association between the RSF complex with centric chromatin, which occurs in mid-G1. This study also highlights the requirement of the RSF complex subunits, Rsf-1 and SNF2h, for the CENP-A maintenance at centromeres.

20. •• Bergmann JH, Rodriguez MG, Martins NM, Kimura H, Kelly DA, Masumoto H, Larionov V, Jansen LE, Earnshaw WC: **Epigenetic engineering shows H3K4me2 is required for HJURP targeting and CENP-A assembly on a synthetic human kinetochore**. *EMBO J* 2010.
Using human artificial chromosomes, the authors show that the presence of H3K4me2 at centric chromatin is required for the recruitment of HJURP, the CENP-A-specific deposition factor, to centromeres and also for the maintenance of centromere identity.

21. • Santenard A, Ziegler-Birling C, Koch M, Tora L, Bannister AJ, Torres-Padilla M-E: **Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3**. *Nat Cell Biol* 2010, **12**:853-862.
The authors illustrate the importance of H3.3 methylation at lysine 27 for *de novo* pericentric heterochromatin formation during early mouse development.

22. Hake SB, Garcia BA, Kauer M, Baker SP, Shabanowitz J, Hunt DF, Allis CD: **Serine 31 phosphorylation of histone variant H3.3 is specific to regions bordering centromeres in metaphase chromosomes**. *Proceedings of the National Academy of Sciences of the United States of America* 2005, **102**:6344-6349.
23. •• Wong LH, Ren H, Williams E, McGhie J, Ahn S, Sim M, Tam A, Earle E, Anderson MA, Mann J, et al.: **Histone H3.3 incorporation provides a unique and functionally essential telomeric chromatin in embryonic stem cells**. *Genome Res* 2009, **19**:404-414.
This study reveals H3.3 at telomeres and centromeres in mECS cells, and illustrates that incorporation of tagged-H3.3 to telomeres is restricted to S phase.

24. •• Goldberg AD, Banaszynski LA, Noh K-M, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, Guo X, Li X, et al.: **Distinct Factors Control Histone Variant H3.3 Localization at Specific Genomic Regions**. *Cell* 2010, **140**:678-691.
This study shows the role of the ATRX-DAXX complex in H3.3 incorporation at telomeres. The authors also show that HIRA is required for H3.3 deposition at active gene promoters and gene bodies in mESCs.

25. •• Drane P, Ouararhni K, Depaux A, Shuaib M, Hamiche A: **The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3**. *Genes & Development* 2010, **24**:1253-1265.
The authors illustrate the importance of the ATRX-DAXX complex in H3.3 localization at pericentric heterochromatin.

26. • Probst AV, Okamoto I, Casanova M, El Marjou F, Le Baccon P, Almouzni G: **A Strand-Specific Burst in Transcription of Pericentric Satellites Is Required for Chromocenter Formation and Early Mouse Development**. *Developmental Cell* 2010, **19**:625-638.
This study highlights the role of strand-specific satellite transcription as an important developmental mechanism for the higher-order organization of pericentric chromatin. The study also showed that blocking replication or interfering with major satellite transcription impedes chromocentre formation during early stages of mammalian development.

27. Jin C, Felsenfeld G: **Nucleosome stability mediated by histone variants H3.3 and H2A.Z**. *Genes Dev* 2007, **21**:1519-1529.
28. • Xu M, Long C, Chen X, Huang C, Chen S, Zhu B: **Partitioning of Histone H3-H4 Tetramers During DNA Replication-Dependent Chromatin Assembly**. *Science* 2010, **328**:94-98.
Using isotope labeling and affinity purification followed by mass spectrometry analysis of histone content, the authors explored how old and new H3-H4 dimers associate with chromatin after DNA replication. They showed that while H3.3-H4 tetramers split *in vivo*, H3.1-H4 tetramers do not.

29. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y: **Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis**. *Cell* 2004, **116**:51-61.
30. • Lewis PW, Elsaesser SJ, Noh K-M, Stadler SC, Allis CD: **Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres**. *Proceedings of the National Academy of Sciences* 2010.
The authors provided with the biochemical characterization of the histone chaperone DAXX.

31. • Law MJ, Lower KM, Voon HPJ, Hughes JR, Garrick D, Viprakasit V, Mitson M, De Gobbi M, Marra M, Morris A, et al.: **ATR-X Syndrome Protein Targets Tandem Repeats and Influences Allele-Specific Expression in a Size-Dependent Manner**. *Cell* 2010, **143**:367-378.
This study defines the genome-wide distribution of ATRX in human and mouse cells and identifies tandem repeats (TR) sequences as the binding sites. These TR sequences are mostly G- and CpG-rich and might form G-quadruplex DNA structures in both hetero- and euchromatin. Furthermore, their study suggests that the number of tandem repeats correlates with disease penetrance and variability.

32. Ritchie K, Seah C, Moulin J, Isaac C, Dick F, Berube NG: **Loss of ATRX leads to chromosome cohesion and congression defects**. *The Journal of Cell Biology* 2008, **180**:315-324.
33. Emelyanov AV, Konev AY, Vershilova E, Fyodorov DV: **Protein Complex of Drosophila ATRX/XNP and HP1a Is Required for the Formation of Pericentric Beta-heterochromatin in Vivo**. *Journal of Biological Chemistry* 2010, **285**:15027-15037.
34. • Wong LH, McGhie JD, Sim M, Anderson MA, Ahn S, Hannan RD, George AJ, Morgan KA, Mann JR, Choo KH: **ATRX interacts with H3.3 in maintaining telomere structural integrity in pluripotent embryonic stem cells**. *Genome Res* 2010, **20**:351-360.
The authors describe the dynamics of ATRX association with heterochromatic landmarks during the cell cycle and ESC differentiation.

35. Ishov AM, Vladimirova OV, Maul GG: **Heterochromatin and ND10 are cell-cycle regulated and phosphorylation-dependent alternate nuclear sites of the transcription repressor Daxx and SWI/SNF protein ATRX**. *J Cell Sci* 2004, **117**:3807-3820.

36. Albert I, Mavrich TN, Tomsho LP, Qi J, Zanton SJ, Schuster SC, Pugh BF: **Translational and rotational settings of H2A.Z nucleosomes across the Saccharomycescerevisiae genome**. *Nature* 2007, **446**:572-576.
37. •• Kim H-S, Vanoosthuyse V, Fillingham J, Roguev A, Watt S, Kislinger T, Treyer A, Carpenter LR, Bennett CS, Emili A, et al.: **An acetylated form of histone H2A.Z regulates chromosome architecture in Schizosaccharomyces pombe**. *Nat Struct Mol Biol* 2009, **16**:1286-1293.
The authors illustrated the role of H2AZ in proper mitotic chromosome condensation in *S. pombe*.

38. •• Zofall M, Fischer T, Zhang K, Zhou M, Cui B, Veenstra TD, Grewal SIS: **Histone H2A.Z cooperates with RNAi and heterochromatin factors to suppress antisense RNAs**. *Nature* 2009, **461**:419-422.
The authors characterized H2AZ genome-wide distribution and deposition machinery in *S. pombe,* and showed that H2AZ cooperates with heterochromatin factors in silencing antisense transcription.

39. Hou H, Wang Y, Kallgren SP, Thompson J, Yates JR, Jia S: **Histone Variant H2A.Z Regulates Centromere Silencing and Chromosome Segregation in Fission Yeast**. *Journal of Biological Chemistry* 2009, **285**:1909-1918.
40. Greaves IK, Rangasamy D, Ridgway P, Tremethick DJ: **H2A.Z contributes to the unique 3D structure of the centromere**. *Proc Natl Acad Sci U S A* 2007, **104**:525-530.
41. • Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR: **Distinctive chromatin in human sperm packages genes for embryo development**. *Nature* 2009, **460**:473-478.
The authors describe the genome-wide localization of histones carrying different posttranslational modifications in mature human sperm.

42. • Brykczynska U, Hisano M, Erkek S, Ramos L, Oakeley EJ, Roloff TC, Beisel C, Schubeler D, Stadler MB, Peters AHFM: **Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa**. *Nat Struct Mol Biol* 2010, **17**:679-687.
See the reference from Hammoud *et al*., 2009.

43. Luk E, Vu N-D, Patteson K, Mizuguchi G, Wu W-H, Ranjan A, Backus J, Sen S, Lewis M, Bai Y, et al.: **Chz1, a Nuclear Chaperone for Histone H2AZ**. *Molecular Cell* 2007, **25**:357-368.
44. Morrison AJ, Shen X: **Chromatin remodelling beyond transcription: the INO80 and SWR1 complexes**. *Nat Rev Mol Cell Biol* 2009, **10**:373-384.
45. •• Buchanan L, Durand-Dubief M, Roguev A, Sakalar C, Wilhelm B, Stralfors A, Shevchenko A, Aasland R, Shevchenko A, Ekwall K, et al.: **The Schizosaccharomyces pombe JmjC-Protein, Msc1, Prevents H2A.Z Localization in Centromeric and Subtelomeric Chromatin Domains**. *PLoS Genet* 2009, **5**:e1000726.
The authors characterized H2AZ genome-wide distribution. They also described the H2AZ deposition complex SpSWR1-C in *S. pombe*, and showed that H2AZ deposition at centromeres and subtelomeric repeats occurs in the absence of *Sp*Swr1, the catalytic subunit of this complex. This contrasts with the localization of H2AZ at other genomic sites.

46. • Papamichos-Chronakis M, Watanabe S, Rando OJ, Peterson CL: **Global Regulation of H2A.Z Localization by the INO80 Chromatin-Remodeling Enzyme Is Essential for Genome Integrity**. *Cell* 2011, **144**:200-213.
This paper shows that the INO80 complex can exchange H2AZ for H2A *in vitro* and *in vivo* and that absence of INO80 causes mislocalization of H2AZ across the genome. The enzymatic activity of INO80 is suggested to remove mislocalized unacetylated H2AZ, which is detrimental for genome integrity.

47. • Luk E, Ranjan A, FitzGerald PC, Mizuguchi G, Huang Y, Wei D, Wu C: **Stepwise Histone Replacement by SWR1 Requires Dual Activation with Histone H2A.Z and Canonical Nucleosome**. *Cell* 2010, **143**:725-736.

This paper highlights the mechanism of H2A/H2AZ histone replacement by the SWR1 complex, showing that complex activation depends on the presence of H2A-containing nucleosomes and also free H2AZ/H2B dimers. The authors also describe the genome-wide distribution of homotypic and heterotypic H2AZ-containing nucleosomes and specifically characterize the homo- and heterotypic distribution of H2A and H2AZ nucleosomes at *S. cerevisiae* promoters.

48. • Choi J, Heo K, An W: **Cooperative action of TIP48 and TIP49 in H2A.Z exchange catalyzed by acetylation of nucleosomal H2A**. *Nucleic Acids Res* 2009, **37**:5993-6007.
The authors identified two H2AZ-assoiated subcomplexes and show that the recombinant ATPases TIP48 and TIP49 are able to exchange H2A for H2AZ *in vitro*.

49. Jha S, Dutta A: **RVB1/RVB2: Running Rings around Molecular Biology**. *Molecular Cell* 2009, **34**:521-533.
50. Folco HD, Pidoux AL, Urano T, Allshire RC: **Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres**. *Science* 2008, **319**:94-97.
51. • Kagansky A, Folco HD, Almeida R, Pidoux AL, Boukaba A, Simmer F, Urano T, Hamilton GL, Allshire RC: **Synthetic heterochromatin bypasses RNAi and centromeric repeats to establish functional centromeres**. *Science* 2009, **324**:1716-1719.
Using a plasmid-based minichromosome system in *S. pombe*, the authors showed that synthetic heterochromatin established by tethering Clr4 in the absence of an active RNAi machinery, is sufficient for the *de novo* formation of CenH3-containing centric chromatin and functional kinetochores.

52. Maggert KA, Karpen GH: **The Activation of a Neocentromere in Drosophila Requires Proximity to an Endogenous Centromere**. *Genetics* 2001, **158**:1615-1628.
53. Lam AL, Boivin CD, Bonney CF, Rudd MK, Sullivan BA: **Human centromeric chromatin is a dynamic chromosomal domain that can spread over noncentromeric DNA**. *Proc Natl Acad Sci U S A* 2006, **103**:4186-4191.
54. Ahmed S, Dul B, Qiu X, Walworth NC: **Msc1 Acts Through Histone H2A.Z to Promote Chromosome Stability in Schizosaccharomyces pombe**. *Genetics* 2007, **177**:1487-1497.
55. Murzina N, Verreault A, Laue E, Stillman B: **Heterochromatin Dynamics in Mouse Cells: Interaction between Chromatin Assembly Factor 1 and HP1 Proteins**. *Molecular Cell* 1999, **4**:529-540.
56. Nakano M, Cardinale S, Noskov VN, Gassmann R, Vagnarelli P, Kandels-Lewis S, Larionov V, Earnshaw WC, Masumoto H: **Inactivation of a Human Kinetochore by Specific Targeting of Chromatin Modifiers**. 2008, **14**:507-522.
57. Dion MF, Kaplan T, Kim M, Buratowski S, Friedman N, Rando OJ: **Dynamics of replication-independent histone turnover in budding yeast**. *Science* 2007, **315**:1405-1408.
58. Meneghini MD, Wu M, Madhani HD: **Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin**. *Cell* 2003, **112**:725-736.
59. Pillus L: **MYSTs mark chromatin for chromosomal functions**. *Current Opinion in Cell Biology* 2008, **20**:326-333.
60. • Samoshkin A, Arnaoutov A, Jansen LE, Ouspenski I, Dye L, Karpova T, McNally J, Dasso M, Cleveland DW, Strunnikov A: **Human condensin function is essential for centromeric chromatin assembly and proper sister kinetochore orientation**. *PLoS ONE* 2009, **4**:e6831.
The authors show that condensin dysfunction results in CENP-A loading defects, loss of centromere rigidity, and partial inactivation of Aurora B, which illustrates the importance of condensins in proper kinetochore function.

61. Yong-Gonzalez V, Wang BD, Butylin P, Ouspenski I, Strunnikov A: **Condensin function at centromere chromatin facilitates proper kinetochore tension and ensures correct mitotic segregation of sister chromatids**. *Genes Cells* 2007, **12**:1075-1090.

62. • Bernad R, Sanchez P, Rivera T, Rodriguez-Corsino M, Boyarchuk E, Vassias I, Ray-Gallet D, Arnaoutov A, Dasso M, Almouzni G, et al.: **Xenopus HJURP and condensin II are requires for CENP-A assembly**. *J Cell Biol* 2011, **192**.
Using the *Xenopus* egg extract system, the authors show the ability of human HJURP to substitute for its *Xenopus* paralogue during CENP-A deposition. Also, by using this system the authors define a specific role for Condensin II for proper retention of CENP-A at centromeres.

63. Corpet A, Almouzni G: **Making copies of chromatin: the challenge of nucleosomal organization and epigenetic information**. *Trends Cell Biol* 2009, **19**:29-41.
64. Okada M, Okawa K, Isobe T, Fukagawa T: **CENP-H-containing Complex Facilitates Centromere Deposition of CENP-A in Cooperation with FACT and CHD1**. *Mol. Biol. Cell* 2009, **20**:3986-3995.
65. Yamagishi Y, Honda T, Tanno Y, Watanabe Y: **Two Histone Marks Establish the Inner Centromere and Chromosome Bi-Orientation**. *Science* 2010, **330**:239-243.
66. • Lefrancois P, Euskirchen G, Auerbach R, Rozowsky J, Gibson T, Yellman C, Gerstein M, Snyder M: **Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing**. *BMC Genomics* 2009, **10**:37.
Using ChIP-Seq combined with advanced DNA sequencing the authors showed that in *S. cerevisiae* Cse4p is strongly bound to all centromeres, but also to 132 novel non-centromeric regions, which may represent regions of high histone turnover.

67. Camahort R, Shivaraju M, Mattingly M, Li B, Nakanishi S, Zhu D, Shilatifard A, Workman JL, Gerton JL: **Cse4 Is Part of an Octameric Nucleosome in Budding Yeast**. *Molecular Cell* 2009, **35**:794-805.
68. Zeitlin SG, Baker NM, Chapados BR, Soutoglou E, Wang JYJ, Berns MW, Cleveland DW: **Double-strand DNA breaks recruit the centromeric histone CENP-A**. *Proceedings of the National Academy of Sciences* 2009, **106**:15762-15767.
69. Zeitlin SG, Shelby RD, Sullivan KF: **CENP-A is phosphorylated by Aurora B kinase and plays an unexpected role in completion of cytokinesis**. *The Journal of Cell Biology* 2001, **155**:1147-1158.
70. Andersen JS, Lam YW, Leung AKL, Ong S-E, Lyon CE, Lamond AI, Mann M: **Nucleolar proteome dynamics**. *Nature* 2005, **433**:77-83.
71. Wiedemann SM, Mildner SN, Bonisch C, Israel L, Maiser A, Matheisl S, Straub T, Merkl R, Leonhardt H, Kremmer E, et al.: **Identification and characterization of two novel primate-specific histone H3 variants, H3.X and H3.Y**. *J Cell Biol* 2010, **190**:777-791.
72. Schenk R, Jenke A, Zilbauer M, Wirth S, Postberg J: **H3.5 is a novel hominid-specific histone H3 variant that is specifically expressed in the seminiferous tubules of human testes**. *Chromosoma* 2011:1-11.
73. Cowell IG, Sunter NJ, Singh PB, Austin CA, Durkacz BW, Tilby MJ: **gammaH2AX Foci Form Preferentially in Euchromatin after Ionising-Radiation**. *PLoS ONE* 2007, **2**:e1057.
74. Greaves IK, Rangasamy D, Devoy M, Marshall Graves JA, Tremethick DJ: **The X and Y Chromosomes Assemble into H2A.Z, Containing Facultative Heterochromatin, following Meiosis**. *Molecular and Cellular Biology* 2006, **26**:5394-5405.
75. • Dejardin J, Kingston RE: **Purification of proteins associated with specific genomic Loci**. *Cell* 2009, **136**:175-186.
Using ChIP-Seq combined with advanced DNA sequencing the authors showed that in *S. cerevisiae* Cse4p is strongly bound to all centromeres, but also to 132 novel non-centromeric regions, which may represent regions of high histone turnover.

76. Foltz DR, Jansen LE, Black BE, Bailey AO, Yates JR, 3rd, Cleveland DW: **The human CENP-A centromeric nucleosome-associated complex**. *Nat Cell Biol* 2006, **8**:458-469.
77. Hoyer-Fender S, Costanzi C, Pehrson JR: **Histone MacroH2A1.2 Is Concentrated in the XY-Body by the Early Pachytene Stage of Spermatogenesis**. *Experimental Cell Research* 2000, **258**:254-260.
78. Bernstein E, Muratore-Schroeder TL, Diaz RL, Chow JC, Changolkar LN, Shabanowitz J, Heard E, Pehrson JR, Hunt DF, Allis CD: **A phosphorylated subpopulation of the histone variant macroH2A1 is excluded from the inactive X chromosome and**

enriched during mitosis. *Proceedings of the National Academy of Sciences* 2008, **105**:1533-1538.
79. Baarends WM, Wassenaar E, Hoogerbrugge JW, Schoenmakers S, Sun Z-W, Grootegoed JA: **Increased phosphorylation and dimethylation of XY body histones in the Hr6b-knockout mouse is associated with derepression of the X chromosome**. *Journal of Cell Science* 2007, **120**:1841-1851.

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

	TSH2B Mammals[f]	*Mm, Hs*: TSH2B	Global (sperm) [7] Telomeres (somatic cells) [75]	NA
	H2BFWT Mammals	*Ms: H2BL1* *Hs*: H2BWT	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	*Sp*Mis16	General chaperone for H3-H4 and potentially $CenH3^{CENP-A}$. Might alter chromatin acetylation status	ND	[14,17]
Priming/licensing	Mis18α/β	*Sp*Mis18	Part of the hMis18 complex, which does not associate with $CenH3^{CENP-A}$ *in vivo*	Late anaphase/ telophase → early G1	[14]
Priming/licensing	M18BP1/HsKNL2	*Ce*KNL-2	Part of the hMis18 complex, which does not associate with $CenH3^{CENP-A}$ *in vivo*	Late anaphase/ telophase → early G1	[14]
Deposition *in vitro* & *in vivo*	HJURP	*Sc*Scm3 *Sp*Scm3 *Xl*HJURP	$CenH3^{CENP-A}$ specific chaperone	Late telophase → early G1	[15-17]
Deposition	FACT complex (SSRP1, SPT16)	*DmSsrp1* *Sp*Pob3 *ScSpt16* *Sp*Spt16 *Dm*Dre4	General chaperone for H3-H4, H2A-H2B, and potentially for $CenH3^{CENP-A}$	Constitutive	[64]
Deposition	CHD1	*Sp*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 / (*Sp*SPAC4F10.12) CENP-M (*Sp*Mis17) CENP-N (*Sp*Mis15) CENP-S CENP-T CENP-H / (*Sp*Sim4) CENP-I / (*Sp*Mis6) CENP-K	Multiprotein complex required for centromere specification and kinetochore assembly	Constitutive	[8]
Maintenance	RSF complex (Rsf-1, SNF2h)	*DmRsf-1,* *Sc*Isw1	ATP-dependent chromatin remodeling complex	Mid-G1 → 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.

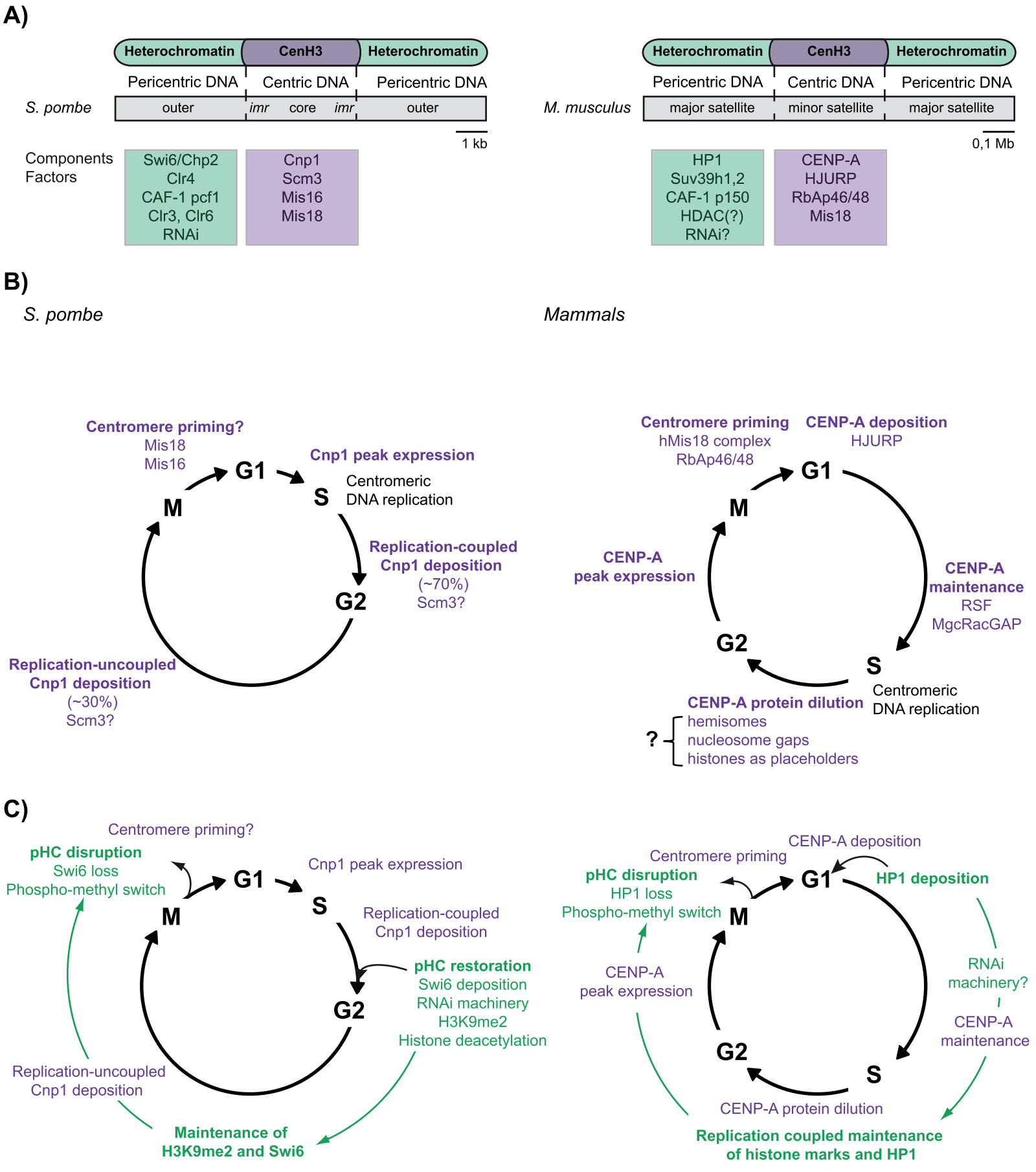
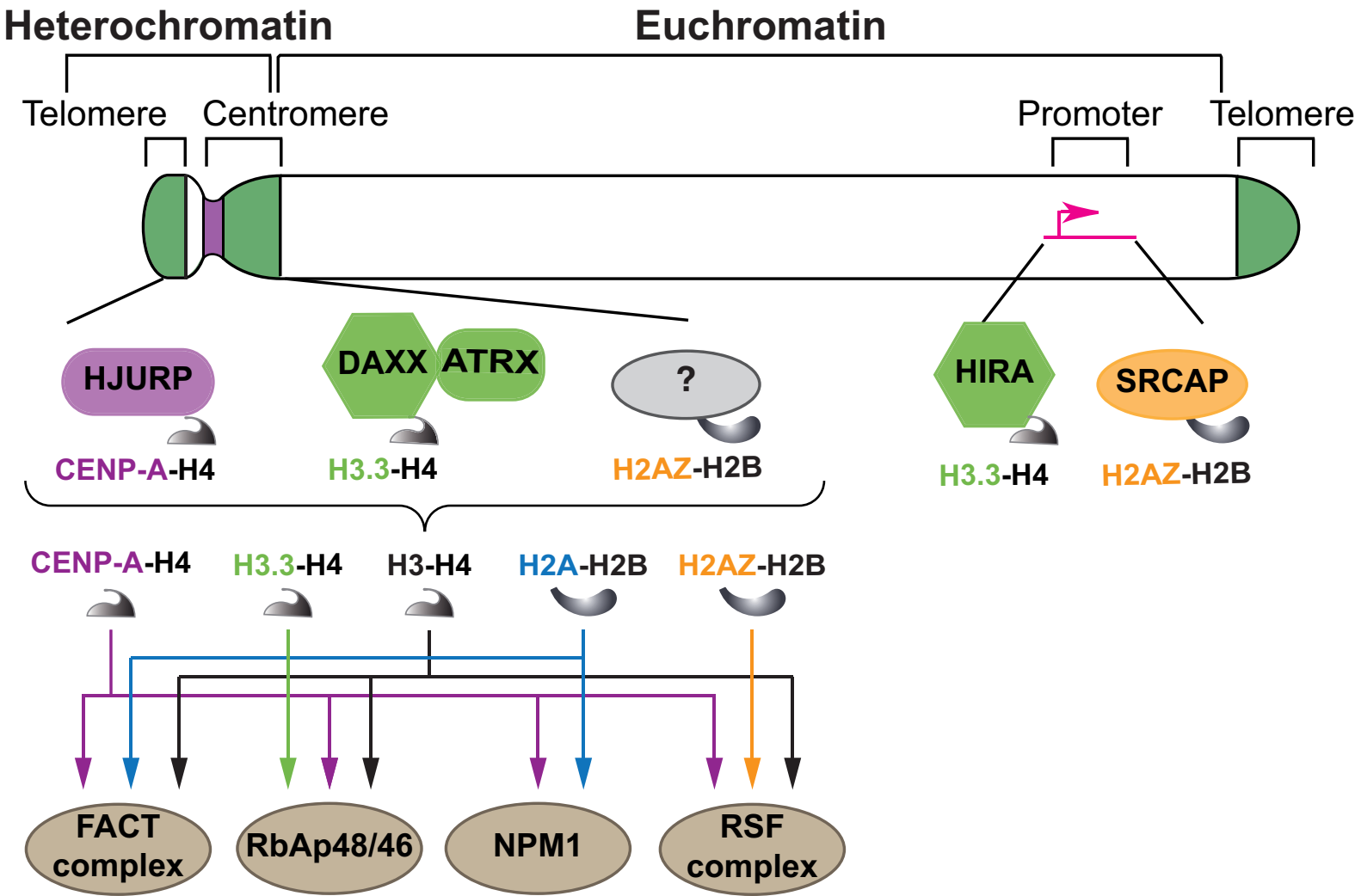

A)
Heterochromatin
CenH3
Heterochromatin
Pericentric DNA
Centric DNA
Pericentric DNA
S. pombe
outer
imr
core
imr
outer
1 kb
M. musculus
major satellite
minor satellite
major satellite
0,1 Mb
Components
Factors
Swi6/Chp2
Clr4
CAF-1 pcf1
Clr3, Clr6
RNAi
Cnp1
Scm3
Mis16
Mis18
HP1
Suv39h1,2
CAF-1 p150
HDAC(?)
RNAi?
CENP-A
HJURP
RbAp46/48
Mis18
B)
S. pombe
Mammals
Centromere priming?
Mis18
Mis16
G1
Cnp1 peak expression
S
Centromeric
DNA replication
M
G2
Replication-coupled
Cnp1 deposition
(~70%)
Scm3?
Replication-uncoupled
Cnp1 deposition
(~30%)
Scm3?
Centromere priming
hMis18 complex
RbAp46/48
CENP-A deposition
HJURP
G1
M
CENP-A
peak expression
CENP-A
maintenance
RSF
MgcRacGAP
G2
S
Centromeric
DNA replication
CENP-A protein dilution
?
hemisomes
nucleosome gaps
histones as placeholders
C)
Centromere priming?
pHC disruption
Swi6 loss
Phospho-methyl switch
G1
Cnp1 peak expression
M
S
Replication-coupled
Cnp1 deposition
pHC restoration
Swi6 deposition
RNAi machinery
H3K9me2
Histone deacetylation
G2
Replication-uncoupled
Cnp1 deposition
Maintenance of
H3K9me2 and Swi6
CENP-A deposition
Centromere priming
pHC disruption
HP1 loss
Phospho-methyl switch
G1
HP1 deposition
M
CENP-A
peak expression
RNAi
machinery?
CENP-A
maintenance
G2
S
CENP-A protein dilution
Replication coupled maintenance
of histone marks and HP1

Figure 2.


Heterochromatin
Euchromatin
Telomere
Centromere
Promoter
Telomere
HJURP
CENP-A-H4
DAXX
ATRX
H3.3-H4
?
H2AZ-H2B
HIRA
H3.3-H4
SRCAP
H2AZ-H2B
CENP-A-H4
H3.3-H4
H3-H4
H2A-H2B
H2AZ-H2B
FACT complex
RbAp48/46
NPM1
RSF complex