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Heterochromatin maintenance and establishment: lessons from the mouse pericentromere

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Abstract

Defined as a chromatin structure that remains condensed throughout the cell cycle heterochromatin is generally transcriptionally silent and is characterized by a specific molecular signature. Constitutive heterochromatin at the pericentromere is a conserved feature throughout evolution, which impacts genome stability. Here, we will summarize recent advances in our understanding of the dynamics of mouse pericentric heterochromatin during the cell cycle and development. Comparison with heterochromatin maintenance in fission yeast will enable discussions of the common basic principles and various mechanisms exploited in the distinct organisms.

Words: 83

Introduction

Initially discovered based on differential staining with DNA dyes, heterochromatin is defined as chromosomal domains that, in contrast to euchromatin, remain condensed throughout the cell cycle¹. Studies in different model organisms have identified heterochromatin regions close to centromeres and telomeres, which make up constitutive heterochromatin². These forms of heterochromatin are critical for genome maintenance and are considered to be vital for repression of transposable elements. Especially, heterochromatin that forms at repetitive

elements at the pericentromeres has been characterized extensively and emerged as the paradigm for constitutive heterochromatin. Here, we will summarize our knowledge on pericentric heterochromatin establishment and dynamics during murine development. We will also compare the features of pericentric heterochromatin in fission yeast and mice to illustrate possible common principles of heterochromatin maintenance.

While formation of a repressive chromatin environment at pericentric repeats is evolutionarily conserved, in different organisms distinct combinations of chromatin marks characterize pericentric heterochromatin at the molecular level. These chromatin marks include DNA methylation, specific histone variants, hypoacetylation and repressive histone modifications, in particular H3K9 methylation, which constitutes a binding site for the Heterochromatin protein 1 (HP1) family. A shared element, whose exact importance remains to be elucidated, is the clustering of pericentric heterochromatin into chromocenters in interphase nuclei (Figure 1). This spatial arrangement, which can compartmentalize heterochromatin away from the rest of the genome, might serve to create a local environment where silencing factors could possibly concentrate and help maintain the heterochromatin status. In fission yeast, the three chromosomes bring their centromeres together in a single chromocenter³ while in mouse as well as in certain plants such as *Arabidopsis*, the arrangement of the chromosomes into subgroups in interphase gives rise to several chromocenters^{4, 5}. Interestingly, chromocenter organization is globally maintained even through S-phase⁶ and dissociation in individual chromosomes is only observed during mitosis. However, this organization can differ in distinct cell types and developmental stages as has been documented in mouse⁷⁻¹¹. This demonstrates the fact that chromocenter organization is dynamic during development; it is established early during development, and depending on the cell type and the state of differentiation different numbers of chromocenters are formed.

Maintenance of heterochromatin marks throughout the cell cycle

Studies in *S. pombe* have been instrumental in gaining insight into the mechanisms in place in order to ensure the maintenance of heterochromatic features during the cell cycle. At fission yeast centromeres, heterochromatin forms at repetitive DNA elements, referred to as pericentric *dg* and *dh* repeats (Figure 1) and is essential for proper loading of the centromere specific H3-variant Cenp-A and kinetochore assembly¹². Nucleosomes at the pericentric repeats are hypoacetylated and enriched in H3K9me2, a histone mark catalyzed by the histone methyltransferase Clr4 and bound by chromodomain-containing proteins Chp1 and

the HP1 homologues Swi6 and Chp2, which have the capacity to multimerize. Ctr4 itself can bind H3K9me2 via its proper chromodomain and methylate neighboring nucleosomes thereby creating additional binding sites for HP1 proteins. Hypoacetylation of histones is ensured by histone de-acetylases recruited by the HP1 homologs¹³. Together these chromatin marks limit Pol II occupancy and ensure the transcriptionally repressed state at pericentric heterochromatin. Interestingly, expression of Forward and Reverse pericentric transcripts is differentially regulated: Forward transcripts accumulate both in strains deficient in Swi6 and RNAi pathway components, while Reverse transcripts accumulate only in RNAi mutants. This suggests that expression of the Forward strand is under transcriptional and the Reverse strand under post-transcriptional control¹⁴. However, also in wild type cells, an exquisite equilibrium exists between transcriptional silencing and activating activity. Indeed, low-level, bidirectional transcription of the pericentric repeats is actually required to ensure the stable maintenance of molecular heterochromatin marks during the cell cycle. In wild type cells, transcription of pericentric repeats peaks in S-phase. Transcription during this specific time window of the cell cycle could possibly occur as a consequence of the phospho-methyl switch in mitosis which leads to Swi6 delocalization and the dilution of repressive histone marks at the daughter strands^{15, 16}, Figure 2A. Pericentric transcripts are thought to form double stranded RNA or hairpin structures¹⁷, which are further processed by the RNAi machinery¹⁴. The resulting small RNAs (siRNAs) that accumulate transiently in S-phase then target the RNA-induced transcriptional silencing (RITS) complex¹⁸ and Ctr4 to re-establish heterochromatin features. The RNA-directed RNA polymerase complex (RDRC) ensures amplification of the siRNA signal. However, in the absence of the RDRC, small degradation products of pericentric transcripts can mediate H3K9 methylation¹⁹. Taken together, these data suggest that the continuous presence of heterochromatin at pericentric repeats actually requires re-establishment of heterochromatin features after each round of replication. Collectively, work in fission yeast has revealed a critical role for pericentric transcripts and the RNAi-dependent pathway in cell cycle maintenance of heterochromatin in locations where heterochromatin was already established.

In mice, the AT-rich major satellites are arranged in arrays up to several megabases in length²⁰. These highly methylated repeats that are organized into hypoacetylated chromatin show high H3K9 methylation that depends on the Suv39h1/2 methyltransferases and are bound by the three isoforms of HP1. Spreading of heterochromatin features is thought to exploit a self-sustaining loop mechanism through HP1 self-association and interaction with the

H3K9HMTase, Suv39h1/2, which can add more H3K9me^{21, 22}, and the capacity of HP1 to recruit *de novo* methyltransferase activity²³. Observations of reduced DNA methylation at pericentric repeats observed in Suv39h mutant cells supports this model²³. In both *S. pombe* and mouse pericentromeres, H3K9HMTase, working as the writer, together with the reader, HP1/Swi6, propagate the heterochromatin state. However, a role for RNA-based mechanisms in the maintenance of heterochromatin features has remained difficult to establish in mammals²⁴⁻²⁶. In mammals, pericentric repeats are transcribed^{23, 27} and regulated in a cell-cycle dependent manner²⁸, yet, during the cell cycle, transcripts accumulate in mitosis and in G1/S. In contrast, in fission yeast, transcription ceases in mid S-phase when pericentric heterochromatin replicates, and therefore, the analogy to fission yeast cannot be extended to include the exact timing during the cell cycle. Furthermore, if RNAi-based mechanisms play a role, as reported for the transposon-control in oocytes²⁹ the mechanism possibly operates without amplification of the siRNA signal, since a properly defined RNA-directed RNA polymerase still remains to be characterized. It is also possible that maintenance of molecular heterochromatin marks through chromosome duplication may be ensured by the mutual reinforcement of mechanisms that maintain DNA methylation and inheritance of modified histones as discussed³⁰. In this context, the parental histone mark would be sufficient to re-initiate the whole process of heterochromatinization by recruiting HP1 and in turn the H3K9me methyltransferase. Furthermore, the maintenance methyltransferase Dnmt1 together with the SRA-domain protein Np95³¹ could also ensure methylation maintenance and recruit histone deacetylase activity necessary to remove acetylation marks on the newly incorporated histones^{32, 33}. Np95 shows affinity to hemi-methylated DNA^{34, 35} and interacts with H3 tails methylated at position K9³⁶, thereby linking DNA and histone modifications to ensure Np95's function during replication of pericentric domains.

Notably, the chromatin assembly machinery at the replication fork contributes in a decisive manner to heterochromatin maintenance. The Chromatin Assembly Factor 1 (CAF-1) deposits new H3-H4 in a DNA-synthesis coupled manner³⁷. New histone H3 bound to CAF-1 mono-methylated by SetDB1³⁸ is a favoured substrate for Suv39h, which can then establish H3K9me3 and HP1 binding³⁹. How CAF-1 possibly contributes to HP1 inheritance at the replication fork by transferring parental HP1 to the replicated daughter chromatin remains to be established^{6, 40}, Figure 2B. The recently identified chromatin-remodelling factor, SMARCAD1, which interacts with PCNA, is likely to participate in these dynamic events connecting histone methylation and HP1 recruitment⁴¹. In summary, in mammalian

pericentric regions, a combination of factors act in concert to ensure maintenance of heterochromatin: DNA methylation maintenance and histone de-acetylation are coupled at the replication fork, as well as histone deposition with further post-translational modifications. Thus, given the robustness of these multiple layers involved in the cell cycle maintenance of heterochromatin in mammals, an RNA-dependent mechanism may either: not be necessary at all, or may not be possible to reveal under normal conditions.

Interestingly, plant heterochromatin, similar to mammalian heterochromatin, is enriched in both DNA methylation and H3K9methylation. These two chromatin marks are tightly interconnected; histone methylation depends on DNA methylation^{42, 43} and vice versa⁴⁴. DNA methylation is critical to silence transposable elements and repeated sequences⁴⁵ and its maintenance is ensured by active mechanisms operating at the replication fork. However, plants lack HP1-like proteins at heterochromatin that could allow spreading of heterochromatin marks in a self-reinforcing loop as is proposed to occur in fission yeast and mammals. Instead, small RNA molecules processed from satellite transcripts or transposable elements do reinforce heterochromatin at pericentromeres in *Arabidopsis*⁴⁶. While small RNA molecules do not target histone methylation activity as in yeast, they direct *de novo* DNA methylation activity to homologous sequences through a mechanism termed RNA-directed DNA methylation (RdDM,⁴⁷. In plants, it remains to be established whether transcription of pericentric repeats is particularly increased following passage of the replication fork in order to reinforce DNA methylation maintenance. These examples illustrate that while most of the heterochromatin features are shared among different species, certain heterochromatin marks are specific to a given organism, as is the way their heterochromatin maintenance is coordinated with other chromatin modifications.

Pericentric heterochromatin dynamics during reproductive development

During mammalian germ cell development and gametogenesis genome organization undergoes important changes, and this is clearly visible at pericentric heterochromatin domains. While in mature oocytes, pericentric satellite repeats retain most of the somatic heterochromatin marks with the exception of HP1 α , in sperm, the majority of histones are replaced by protamines or sperm-specific histone variants⁴⁸, therefore requiring *de novo* establishment of most heterochromatic features. Upon fertilization, protamines and the

sperm-specific histone variants are removed ⁴⁹ and a nucleosomal organization is restored. As histone assembly occurs in a DNA-synthesis independent manner, only H3.3 and not the replicative H3 variants are incorporated ⁵⁰. This accentuates the chromatin differences at pericentric satellite repeats between the two parental genomes as paternal pericentric domains also lack H3K9me3, and H4K20me3 ⁵¹(Figure 3). Instead, during the first cleavage stages, alternative repressive chromatin factors, the Polycomb group binding proteins, are enriched at paternal pericentric satellites ⁵². In the zygote, the paternal genome undergoes active global DNA demethylation ⁵³, however some residual DNA methylation is retained at pericentric repeats ⁵⁴ where it could play a role in the establishment of other heterochromatin features.

During the early cleavage stages, the spatial organization of pericentric satellite repeats is particularly dynamic; the pericentric domains reorganize from the gamete-specific organization to form rings around the nucleolar precursor bodies ¹¹. These ring-structures progressively reorganize into chromocenters during the 2-cell stage. During this particular developmental time window, transcripts from pericentric major repeats undergo a burst in transcription reaching levels far above the amount observed in cycling embryonic fibroblasts ⁵⁵. While the repeats were known to be transcribed bi-directionally ²⁷, unexpectedly, their transcription and spatial localization is controlled in a strand-specific manner during early embryonic development ⁵⁵. The Forward strand is transcribed during the S-phase of the second cell cycle and peaks in late S/G2 followed by upregulation of the Reverse strand. Transcription from both strands is then strongly downregulated at the 4-cell stage, when chromocenters have formed, which are faithfully propagated during the following cleavage stages (Figure 3). Interference with transcripts during the 2-cell stage results in embryos that fail to form chromocenters and arrest their development in the G2 phase of the second cell cycle ⁵⁵. These experiments illustrate a critical necessity for pericentric transcription during heterochromatin establishment in early embryonic development. How transcription of the two strands is regulated in time and space and which promoters and transcription factors are necessary to drive satellite transcription specifically during this developmental transition are still unresolved questions. Differences in timing of transcription and differential regulation of subsets of repeats by distinct silencing pathways have been observed in fission yeast ^{14, 15} and plants ⁴⁶. Investigating the strand-specific expression of major satellite sequences in mutant backgrounds for chromatin modifiers may help to better understand their mode of regulation.

The exact mechanism of how pericentric transcripts affect chromocenter formation and development as well as the role of the two respective transcripts remain to be dissected, however, recent data have allowed speculation about their mode of action. Fertilized eggs expressing a mutated form of H3.3 (K27R) arrest at the 2-cell stage and show HP1 β mis-localization. This arrest, in addition to proper HP1 β localization can be rescued by injection of double-stranded, but not single stranded major RNA, implying processing of double stranded RNA molecules and a role for small RNA based mechanisms ⁵⁶. Alternatively, pericentric transcripts could function in recruiting heterochromatin components or modifiers, similar to the long non-coding transcript Air, involved in imprinting that interacts with the histone methyltransferase G9a ⁵⁷. An intriguing candidate to interact with pericentric transcripts is HP1, which has RNA-binding capacity ^{58, 59}. HP1 indeed binds major satellite RNA, however, it does so only when sumoylated and it also shows specificity for Forward transcripts. Interestingly, sumoylation of HP1 enhances its capacity to be recruited *de novo* to pericentric satellites lacking H3K9me3 and HP1 ⁶⁰.

In short, the specific organization of DNA in the sperm and the loss of somatic heterochromatin marks entail a challenge for the early embryo to cope with two distinct states of pericentric heterochromatin in the same nucleus. Early development therefore necessitates setting most somatic heterochromatin marks at paternal and requires consolidation of maternal heterochromatin domains; it may also be one of the rare situations in which *de novo* formation of constitutive heterochromatin can be studied in mammals. The demonstration of a critical role of pericentric satellite transcripts may be one of the first steps towards a better understanding of the mechanisms implicated in mammalian heterochromatin establishment.

Figure legends:

Figure 1:

Pericentric heterochromatin clustering

A. 4',6-diamidino-2-phenylindole (DAPI)-stained images and schematic representations of mouse nuclei in interphase (top) or chromosomes in mitosis (bottom). Individual pericentric heterochromatin domains (red) from distinct chromosomes cluster together in chromocenters

during interphase. Scale bar, 10 μ m. Image is reproduced, with permission, from Maison et al, Nat Rev Mol Cell Biol, vol. 5 (4) pp. 296-304 © (2004) Macmillan Publishers Ltd. All rights reserved. B. Clustering of pericentric heterochromatin is a recurrent feature of nuclear organization in a number of organisms independent of the size of their genomes and the number of chromosomes. While in *S. pombe* the three centromeres gather to a single cluster³, in both mouse and *Arabidopsis*, heterochromatin organizes in several chromocenters^{4, 5}. Pericentric heterochromatin nucleates at repetitive elements. In *S. pombe* centromeres, these consist of the outer *dg* and *dh* repeats present on each chromosome. In *Arabidopsis*, heterochromatin forms on 180bp satellite repeats interspersed with Athila retrotransposons and their remnants as well as various types of transposons that flank these repeats^{46, 61}. In mouse, the pericentric domains consist of large uninterrupted arrays of AT-rich 234bp-long major satellite repeats²⁰, which can extend to more than 2 Mb per chromosome.

Figure 2:

Pericentric heterochromatin maintenance during the cell cycle in S. pombe and mouse

A. Fission yeast spends most of its lifetime in G2 phase, during which time, H3K9me2, at pericentric repeats constitutes a binding site for the chromodomain protein Swi6, the HP1 homolog. Phosphorylation of serine 10 of H3 (phospho-methyl switch) during mitosis (M) induces loss of Swi6 binding. This, together with the subsequent dilution of repressive histone modifications upon replication of pericentric repeats in early S-phase creates a small time window during which Pol II gains access and pericentric transcripts accumulate. These transcripts are processed by the RNAi machinery into small RNAs (siRNAs) that target the methyltransferase Clr4 (the homolog of mammalian SUV39H) to pericentric repeats. After deacetylation of the newly incorporated histones, Clr4 then remethylates K9, Swi6 is recruited, and transcriptional silencing is reestablished.

B. In G2, pericentric heterochromatin is enriched in DNA methylation and H3K9me3 that is bound by HP1 in mice. While HP1 binding is affected by the phospho-methyl switch in mitosis^{62, 63}, the extent of HP1 disruption and the mechanisms of its restoration in G1 phase are as yet unknown. Transcripts from pericentric satellites have been observed in mitosis and in G1/early S-phase, however major satellite transcription is downregulated during mid S-phase when pericentric domains replicate²⁸. Coupled to DNA replication, histone de-

acetylase activity is recruited by the DNA clamp PCNA and the DNA methyltransferase Dnmt1³² to deacetylate the newly incorporated histones. Dnmt1 restores DNA methylation. PCNA further recruits the Chromatin Assembly Factor 1 (CAF1). CAF1 can assemble H3 already monomethylated at K9 by SetDB1³⁸ into nucleosomes, but it also plays an important role in HP1 dynamics by transferring parental HP1 to daughter strands⁴⁰.

Figure 3.

Heterochromatin organization and transcription during early cleavage stages in mouse

Mouse sperm DNA tightly compacts around a core of pericentric repeats (blue) in the centre of the sperm¹⁰. After fertilization, the egg completes meiosis II and extrudes one haploid genome in form of the polar body (PB). Two independent pronuclei form, in which pericentric domains reorganize into ring-structures around the nucleolus-like bodies¹¹. Maternal (pink) and paternal (blue) pericentric domains are epigenetically distinct in the zygote^{11, 51, 52} and retain asymmetry up to the 8-cell stage^{52, 64}. Paternal pericentric repeats lack H3K9me3 and H4K20me3 and either do not, or only weakly bind HP1 β ^{51, 52}. During the 2-cell stage chromocenters progressively form and are then propagated during further cleavage stages. The first transcription of the zygotic genome (minor zygotic gene activation, ZGA) takes place at the end of first cell cycle, followed by the major ZGA in the 2-cell stage. The formation of chromocenters during the 2-cell stage coincides with a transcriptional burst of pericentric satellites (⁵⁵ see text).

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References:

1. Heitz E. Das Heterochromatin der Moose. *Jahrbuch Wiss Botanik* 1928;762-818.
2. Brown SW. Heterochromatin. *Science* 1966; 151:417-25.
3. Funabiki H, Hagan I, Uzawa S, Yanagida M. Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. *J Cell Biol* 1993; 121:961-76.
4. Guenatri M, Bailly D, Maison C, Almouzni G. Mouse centric and pericentric satellite repeats form distinct functional heterochromatin. *J Cell Biol* 2004; 166:493-505.
5. Fransz P, De Jong JH, Lysak M, Castiglione MR, Schubert I. Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. *Proc Natl Acad Sci U S A* 2002; 99:14584-9.
6. Quivy JP, Roche D, Kirschner D, Tagami H, Nakatani Y, Almouzni G. A CAF-1 dependent pool of HP1 during heterochromatin duplication. *EMBO J* 2004; 23:3516-26.
7. Mayer R, Brero A, von Hase J, Schroeder T, Cremer T, Dietzel S. Common themes and cell type specific variations of higher order chromatin arrangements in the mouse. *BMC Cell Biol* 2005; 6:44.
8. Terranova R, Sauer S, Merckenschlager M, Fisher AG. The reorganisation of constitutive heterochromatin in differentiating muscle requires HDAC activity. *Exp Cell Res* 2005; 310:344-56.
9. Solovei I, Kreysing M, Lanctot C, Kosem S, Peichl L, Cremer T, et al. Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. *Cell* 2009; 137:356-68.
10. Haaf T, Ward DC. Higher order nuclear structure in mammalian sperm revealed by in situ hybridization and extended chromatin fibers. *Exp Cell Res* 1995; 219:604-11.
11. Probst AV, Santos F, Reik W, Almouzni G, Dean W. Structural differences in centromeric heterochromatin are spatially reconciled on fertilisation in the mouse zygote. *Chromosoma* 2007; 116:403-15.
12. Folco HD, Pidoux AL, Urano T, Allshire RC. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. *Science* 2008; 319:94-7.
13. Yamada T, Fischle W, Sugiyama T, Allis CD, Grewal SI. The nucleation and maintenance of heterochromatin by a histone deacetylase in fission yeast. *Mol Cell* 2005; 20:173-85.
14. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 2002; 297:1833-7.
15. Kloc A, Zaratiegui M, Nora E, Martienssen R. RNA Interference Guides Histone Modification during the S Phase of Chromosomal Replication. *Curr Biol* 2008; 18:490-5.
16. Chen ES, Zhang K, Nicolas E, Cam HP, Zofall M, Grewal SI. Cell cycle control of centromeric repeat transcription and heterochromatin assembly. *Nature* 2008; 451:734-7.
17. Djupedal I, Kos-Braun IC, Mosher RA, Soderholm N, Simmer F, Hardcastle TJ, et al. Analysis of small RNA in fission yeast; centromeric siRNAs are potentially generated through a structured RNA. *EMBO J* 2009; 28:3832-44.
18. Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SI, et al. RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* 2004; 303:672-6.
19. Halic M, Moazed D. Dicer-independent primal RNAs trigger RNAi and heterochromatin formation. *Cell* 2010; 140:504-16.

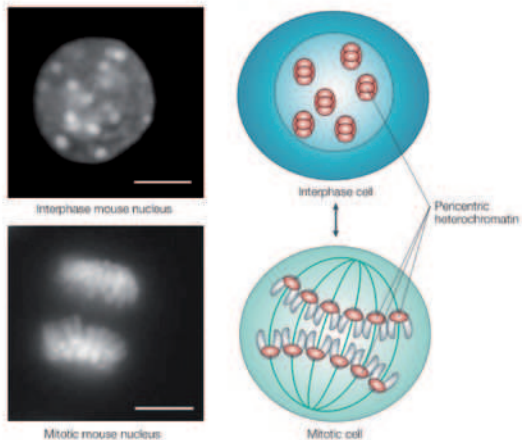
20. Vissel B, Choo KH. Mouse major (gamma) satellite DNA is highly conserved and organized into extremely long tandem arrays: implications for recombination between nonhomologous chromosomes. *Genomics* 1989; 5:407-14.
21. Aagaard L, Laible G, Selenko P, Schmid M, Dorn R, Schotta G, et al. Functional mammalian homologues of the *Drosophila* PEV-modifier Su(var)3-9 encode centromere-associated proteins which complex with the heterochromatin component M31. *EMBO J* 1999; 18:1923-38.
22. Maison C, Almouzni G. HP1 and the dynamics of heterochromatin maintenance. *Nat Rev Mol Cell Biol* 2004; 5:296-304.
23. Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, Kubicek S, et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* 2003; 13:1192-200.
24. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 2005; 19:489-501.
25. Murchison EP, Partridge JF, Tam OH, Cheloufi S, Hannon GJ. Characterization of Dicer-deficient murine embryonic stem cells. *Proc Natl Acad Sci U S A* 2005; 102:12135-40.
26. Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, Godwin J, et al. T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. *J Exp Med* 2005; 201:1367-73.
27. Rudert F, Bronner S, Garnier JM, Dolle P. Transcripts from opposite strands of gamma satellite DNA are differentially expressed during mouse development. *Mamm Genome* 1995; 6:76-83.
28. Lu J, Gilbert DM. Proliferation-dependent and cell cycle regulated transcription of mouse pericentric heterochromatin. *J Cell Biol* 2007; 179:411-21.
29. Stein P, Svoboda P, Anger M, Schultz RM. RNAi: mammalian oocytes do it without RNA-dependent RNA polymerase. *RNA* 2003; 9:187-92.
30. Probst AV, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. *Nat Rev Mol Cell Biol* 2009; 10:192-206.
31. Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* 2007; 450:908-12.
32. Fuks F, Burgers WA, Brehm A, Hughes-Davies L, Kouzarides T. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet* 2000; 24:88-91.
33. Unoki M, Nishidate T, Nakamura Y. ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* 2004; 23:7601-10.
34. Arita K, Ariyoshi M, Tochio H, Nakamura Y, Shirakawa M. Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. *Nature* 2008; 455:818-21.
35. Avvakumov GV, Walker JR, Xue S, Li Y, Duan S, Bronner C, et al. Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature* 2008; 455:822-5.
36. Rottach A, Frauer C, Pichler G, Bonapace IM, Spada F, Leonhardt H. The multi-domain protein Np95 connects DNA methylation and histone modification. *Nucleic Acids Res* 2010; 38:1796-804.
37. Verreault A, Kaufman PD, Kobayashi R, Stillman B. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* 1996; 87:95-104.
38. Loyola A, Tagami H, Bonaldi T, Roche D, Quivy JP, Imhof A, et al. The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. *EMBO Rep* 2009; 10:769-75.

39. Loyola A, Bonaldi T, Roche D, Imhof A, Almouzni G. PTMs on H3 variants before chromatin assembly potentiate their final epigenetic state. *Mol Cell* 2006; 24:309-16.
40. Quivy JP, Gerard A, Cook AJ, Roche D, Almouzni G. The HP1-p150/CAF-1 interaction is required for pericentric heterochromatin replication and S-phase progression in mouse cells. *Nat Struct Mol Biol* 2008; 15:972-9.
41. Rowbotham SP, Barki L, Neves-Costa A, Santos F, Dean W, Hawkes N, et al. Maintenance of Silent Chromatin through Replication Requires SWI/SNF-like Chromatin Remodeler SMARCA1. *Mol Cell* 2011; 42:285-96.
42. Soppe WJ, Jasencakova Z, Houben A, Kakutani T, Meister A, Huang MS, et al. DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in Arabidopsis. *EMBO J* 2002; 21:6549-59.
43. Tariq M, Saze H, Probst AV, Lichota J, Habu Y, Paszkowski J. Erasure of CpG methylation in Arabidopsis alters patterns of histone H3 methylation in heterochromatin. *Proc Natl Acad Sci U S A* 2003; 100:8823-7.
44. Jackson JP, Lindroth AM, Cao X, Jacobsen SE. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 2002; 416:556-60.
45. Rigal M, Mathieu O. A "mille-feuille" of silencing: Epigenetic control of transposable elements. *Biochim Biophys Acta* 2011.
46. May BP, Lippman ZB, Fang Y, Spector DL, Martienssen RA. Differential regulation of strand-specific transcripts from Arabidopsis centromeric satellite repeats. *PLoS Genet* 2005; 1:e79.
47. Wassenegger M, Heimes S, Riedel L, Sanger HL. RNA-directed de novo methylation of genomic sequences in plants. *Cell* 1994; 76:567-76.
48. Govin J, Escoffier E, Rousseaux S, Kuhn L, Ferro M, Thevenon J, et al. Pericentric heterochromatin reprogramming by new histone variants during mouse spermiogenesis. *J Cell Biol* 2007; 176:283-94.
49. Wu F, Caron C, De Robertis C, Khochbin S, Rousseaux S. Testis-specific histone variants H2AL1/2 rapidly disappear from paternal heterochromatin after fertilization. *J Reprod Dev* 2008; 54:413-7.
50. van der Heijden GW, Dieker JW, Derijck AA, Muller S, Berden JH, Braat DD, et al. Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. *Mech Dev* 2005; 122:1008-22.
51. Santos F, Peters AH, Otte AP, Reik W, Dean W. Dynamic chromatin modifications characterise the first cell cycle in mouse embryos. *Dev Biol* 2005; 280:225-36.
52. Puschendorf M, Terranova R, Boutsma E, Mao X, Isono K, Brykczynska U, et al. PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos. *Nat Genet* 2008; 40:411-20.
53. Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. *Nature* 2000; 403:501-2.
54. Santos F, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 2002; 241:172-82.
55. 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. *Dev Cell* 2010; 19:625-38.
56. Santenard A, Ziegler-Birling C, Koch M, Tora L, Bannister AJ, Torres-Padilla ME. Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nat Cell Biol* 2010.
57. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* 2008; 322:1717-20.

58. Muchardt C, Guilleme M, Seeler JS, Trouche D, Dejean A, Yaniv M. Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. *EMBO Rep* 2002; 3:975-81.
59. Maison C, Bailly D, Peters AH, Quivy JP, Roche D, Taddei A, et al. Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nat Genet* 2002; 30:329-34.
60. Maison C, Bailly D, Roche D, Montes de Oca R, Probst AV, Vassias I, et al. SUMOylation promotes de novo targeting of HP1alpha to pericentric heterochromatin. *Nat Genet* 2011; 43:220-7.
61. Kumeoka N, Hosouchi T, Tsuruoka H, Kotani H. The size and sequence organization of the centromeric region of arabidopsis thaliana chromosome 5. *DNA Res* 2000; 7:315-21.
62. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, et al. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature* 2005; 438:1116-22.
63. Hirota T, Lipp JJ, Toh BH, Peters JM. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature* 2005; 438:1176-80.
64. Merico V, Barbieri J, Zuccotti M, Joffe B, Cremer T, Redi CA, et al. Epigenomic differentiation in mouse preimplantation nuclei of biparental, parthenote and cloned embryos. *Chromosome Res* 2007.

FIGURE 1

A.

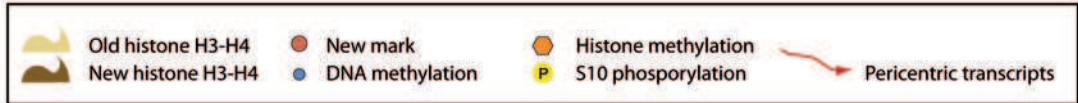
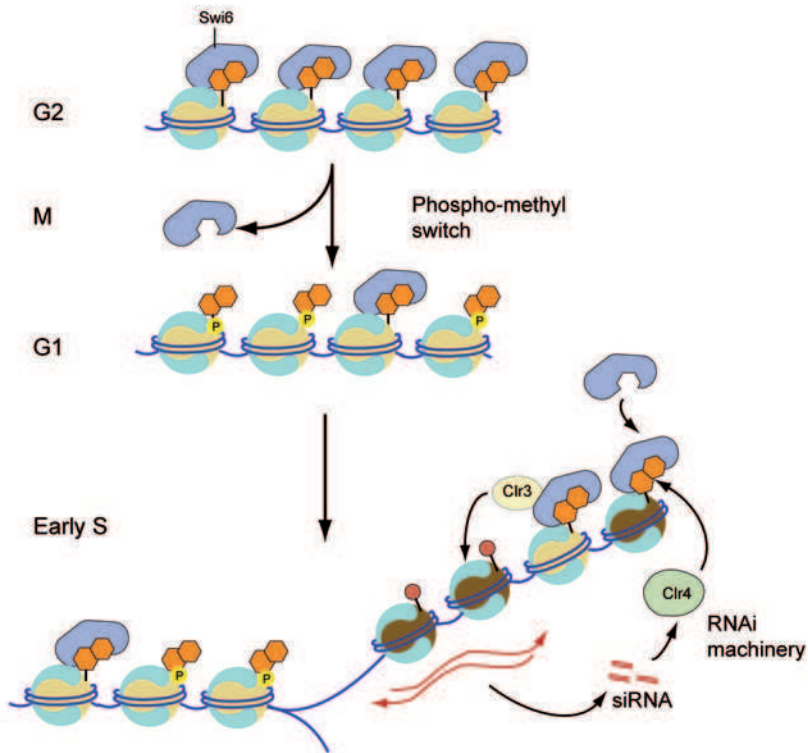


B.

organism	# of chr	chromocenters	repetitive elements
<i>S. pombe</i>	3	1	dg, dh repeats
<i>A. thaliana</i>	5	~8	180bp satellites, Athila retrotransposons
<i>M. musculus</i>	20	~8-30	234bp major satellites

FIGURE 2

A. Fission yeast



B. Mice

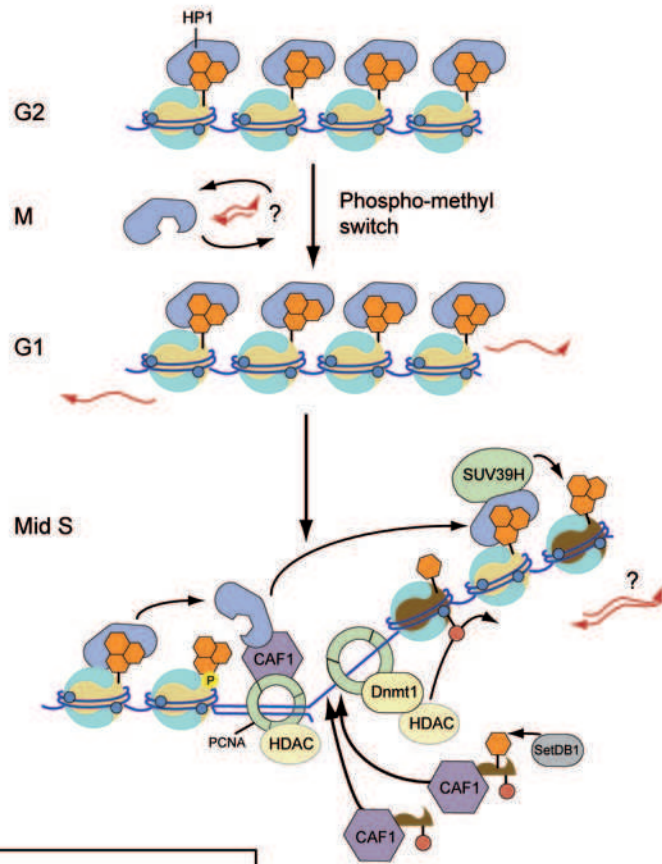


FIGURE 3

