HP1 AND THE DYNAMICS OF HETEROCHROMATIN MAINTENANCE

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Heterochromatin maintenance is crucial for the clonal inheritance of cell identity, to ensure the proper segregation of chromosomes and the regulation of gene expression. Although it is architecturally stable, heterochromatin has to be flexible to cope with disrupting events such as replication. Recent progress has shed light on the paradoxical properties of heterochromatin in the nucleus, and highlights the roles of heterochromatin protein-1 and, more unexpectedly, RNA molecules in heterochromatin maintenance.

EUCHROMATIN
A form of chromatin that is de-condensed during interphase.

KINETOCHORE
A multiprotein complex that assembles on centromeric DNA and mediates the attachment and movement of chromosomes along the microtubules of the mitotic spindle.

CHROMOCENTRE
Aggregates of centromere from different chromosomes.

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In eukaryotic cells, the genetic material is organized into a complex structure that is known as chromatin (from the Greek *khroma* meaning coloured), which was first detected with basic dyes at the end of the nineteenth century[1]. Historically, on the basis of microscopic observations, chromatin is divided into two distinct domains, heterochromatin and EUCHROMATIN. Heterochromatin is defined as regions of chromatin that remain cytologically condensed and densely stained throughout the cell cycle, whereas euchromatin is de-condensed during interphase[2]. Large blocks of heterochromatin surround functional chromosome structures such as centromeres and telomeres. At centromeres, around the centric region that is directly involved in KINETOCHORE formation[3], these blocks are known as pericentric regions. In mouse cells, these regions are easily identified by their intense DNA staining with the dye, 4′,6-diamidino-2-phenylindole (DAPI). They are observed throughout the cell cycle (FIG. 1), and are most visible during interphase when these regions from several chromosomes cluster together as CHROMOCENTRES. So, on the basis of a historical definition, PERICENTRIC HETEROCHROMATIN is a typical example of constitutive heterochromatin, for which potential functional importance for chromosome segregation and gene expression has been documented. Here, we summarize the present knowledge of the properties of pericentric heterochromatin as a basis for our understanding of how a nuclear domain, which is epigenetically marked, can be maintained. This will not only enrich the definition of heterochromatin[4] by introducing the molecular components that are involved in its structure, but also emphasize the unique aspects of pericentric heterochromatin.

Pericentric heterochromatin is composed mainly of long stretches of repetitive A+T-rich DNA sequence, and includes transposable DNA elements and SATELLITE REPEATS that, in mouse cells, are known as the major satellite repeats. These satellite repeats consist of a 234-bp monomer that is tandemly arranged into higher-order arrays that extend over a length ranging from 240 kb to greater than 2,000 kb (REF. 5). Given its position — juxtaposed to centric regions (FIG. 1) — it is not surprising that the proper organization of pericentric heterochromatin can be vital in ensuring correct chromosome segregation[6,7] and therefore has an important role in the maintenance of genome stability, as was initially found in the fission yeast *Schizosaccharomyces pombe*[8]. In addition, pericentric heterochromatin has been implicated in the gene silencing that occurs when euchromatic genes are placed adjacent to heterochromatin by chromosome rearrangement or transposition[9,10] — a phenomenon that was initially described in *Drosophila melanogaster* as POSITION-EFFECT VARIEGATION[11].

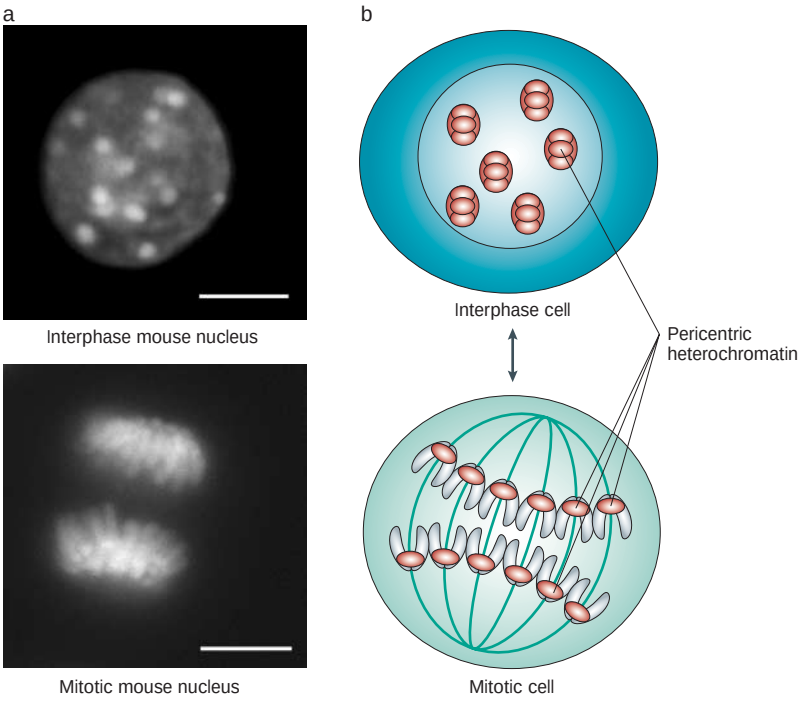


Figure 1 | **Mouse pericentric heterochromatin.** Images of nuclei from interphase (top) and mitotic (bottom) mouse cells that are stained with 4′,6-diamidino-2-phenylindole (DAPI) are shown (**a**) along with schematic representations (**b**). In interphase cells, the clustering of regions that correspond to pericentric heterochromatin from different chromosomes give rise to the bright spots (or chromocentres). In mitotic cells, the chromosome clustering is disrupted, and each spot corresponds to pericentric-heterochromatin domains from individual chromosomes. Scale bars, 10 μm.

PERICENTRIC HETEROCHROMATIN
A region of chromatin that is found juxtaposed to the centromere and that remains condensed throughout the cell cycle. It is considered to be typical constitutive heterochromatin.

SATELLITE REPEAT
A specific DNA sequence that is repeated many times in a long tandem array.

POSITION-EFFECT VARIEGATION
The heritable suppression of genes that results from their abnormal translocation to a position close to heterochromatin.

CHROMODOMAIN
A highly conserved protein-sequence motif that is common to many chromosomal proteins. In HP1 it is crucial for the interaction with histone H3 that is methylated at lysine 9.

CHROMOSHADOW DOMAIN
A protein-sequence motif that is related to the chromodomain in its amino-acid sequence and has so far only been found in the HP1 family of proteins.

Importantly, in addition to the repetitive-DNA content that is mitotically inherited, pericentric heterochromatin has several 'marks' that are stably transmitted across cell generations, and therefore, by definition, are of an epigenetic nature. In mouse cells, DNA is methylated at CpG dinucleotides[12]. At the nucleosomal level, modified histones are present, generally hypoacetylated[13] and specifically methylated at lysine 9 of histone H3 (H3-K9)[7]. This latter modification is dependent on histone-H3 methyltransferases that are encoded by homologues of the *D. melanogaster Suppressor of variegation 3-9* gene — they are known as *Suv39h1* and *Suv39h2* in the mouse[7]. Finally, a prominent mark in this region is the enrichment in heterochromatin protein-1 (HP1)[14].

In this article, HP1 is reviewed and its binding partners and dynamic properties are discussed. The mechanisms for the maintenance of heterochromatin organization through HP1 at pericentric loci are presented. We also speculate on a possible mode for HP1 inheritance during replication. Finally, on the basis of exciting recent discoveries, we compare the possible functions of RNA at pericentric heterochromatin in mouse cells and *S. pombe*.

Binding of HP1 to heterochromatin

HP1 protein organization. HP1 was initially identified in *D. melanogaster* in a screen for nuclear proteins that showed a predominant distribution to the chromocentre on polytene chromosomes[14,15]. Genetic analysis in *D. melanogaster* further implicated HP1 in the regulation of gene expression by position-effect variagation[16]. This highly conserved protein has homologues in various organisms, ranging from *S. pombe* (Swi6) to mammals, in which three HP1 isoforms, HP1α, HP1β (also known as MOD1 or M31) and HP1γ (also known as MOD2 or M32) have been identified[17]. Although the HP1 isoforms have similarities in their amino-acid sequences and structural organization (FIG. 2), there are some differences in their localization. They are primarily associated with centromeric heterochromatin, but HP1β and, in particular, HP1γ also localize to euchromatic sites[18,19]. The specificity of HP1α for pericentromeric regions has gained further support recently[20]. HP1 proteins are small (around 25 kDa) and contain a conserved amino-terminal region that is known as the CHROMODOMAIN (chromatin-organization modifier), followed by a variable hinge region and a conserved carboxy-terminal CHROMOSHADOW DOMAIN (FIG. 2). In general, chromodomains are found in numerous proteins, many of which are known to function in chromatin organization and the regulation of gene expression[21], such as Polycomb proteins (which repress transcription of many loci in the genome) and Suv39h1. The three-dimensional structure of both the chromodomain and the chromoshadow domain of mouse HP1β, which was determined in solution using NMR, shows that each domain forms a hydrophobic pocket and can function as a protein-binding motif[22–24]. The crystal structure of the chromoshadow domain of Swi6 in *S. pombe* has been solved[25], as well as the co-crystal structure of modified H3 peptide with the chromodomain of *D. melanogaster* HP1 (REF. 26). The multipartite organization of HP1 proteins allows several proteins to bind simultaneously and has led to the idea that HP1 proteins can function as structural adaptors that are vital for the assembly of macromolecular complexes in chromatin.

HP1-binding partners and heterochromatin stability. Among the many nuclear proteins with which mouse HP1α can interact[27], we focus here on those that have a potential role in the stability of the higher-order structure of pericentric heterochromatin (FIG. 2).

For the chromodomain of mouse HP1α, the most prominent binding partner is histone H3 that is methylated at lysine 9 (H3-K9; REFS 28,29), a property that has been proposed to be consistent with the HISTONE-CODE HYPOTHESIS[30–32]. Lysine residues can be mono-, di-, and trimethylated *in vivo*, and recently a detailed analysis showed that HP1 has a preferred affinity for a histone-H3 peptide that bears trimethylated K9 (REF. 26). Interestingly, given that Suv39h1 can introduce this K9 modification and is also able to bind HP1 (REF. 33), the idea emerged that the propagation of heterochromatin can be ensured once an initiating site has been established. This propagation would involve a 'self-sustaining' loop, in which methylated H3-K9 histones bind to HP1; which in turn recruits more H3-K9 histone methyltransferase[34]. This type of mechanism could potentially apply to other histone modifications.

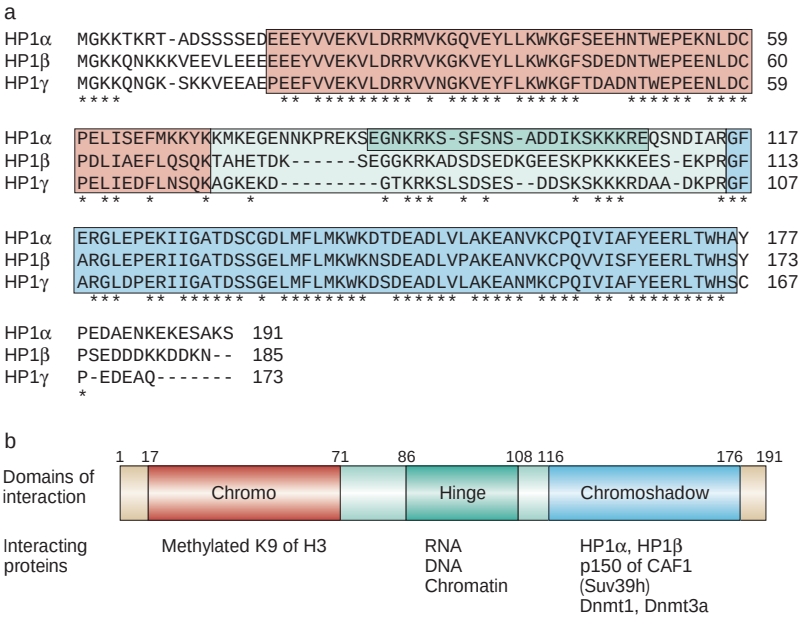


Figure 2 | **Mouse HP1 domains and interaction partners. a** | The amino-acid sequence of mouse heterochromatin protein-1 (HP1) isoforms α, β and γ. Asterisks indicate amino acids that are conserved among the three subtypes. Three domains are distinguished: the amino-terminal chromodomain (red), the central hinge region (pale green) and the chromoshadow domain (blue). The RNA-binding activity of HP1α is mediated by a domain of 23 amino acids (mid green) that is located in the carboxy-terminal part of the hinge region. **b** | This scheme depicts HP1α domains of interaction with selected partners that are potentially important for the stability of heterochromatin domains. The chromodomain binds to methylated lysine 9 of histone H3. The hinge region can bind RNA, DNA and chromatin. Among the chromoshadow-domain interactors are HP1α and HP1β, p150 of chromatin-assembly factor-1 (CAF1), the histone H3 lysine methyltransferase Suv39h (this interaction was shown in *Drosophila melanogaster*) and the DNA methyltransferases Dnmt1 and Dnmt3a.

Interestingly, the *D. melanogaster* histone acetyltransferase Mof (males absent on the first), which is involved in male X-chromosome DOSAGE COMPENSATION, contains a chromodomain that functions as a protein–RNA-binding module. *In vivo*, the association of Mof with the male X chromosome was therefore proposed to depend on the interaction of Mof with *roX2* RNA[35]. However, there is poor sequence similarity between the chromodomain of Mof and HP1. So, although the enrichment in HP1 proteins at centromeric regions is sensitive to RNase in mouse cells[36], and HP1 proteins can bind RNA *in vitro*[37], this binding does not involve the HP1 chromodomain. Unlike Mof, the RNA-binding properties of HP1α are mediated *in vitro* by the hinge region[37], a region with no obvious homology to known RNA-binding proteins. In addition, the hinge region of HP1 can also bind DNA and chromatin without obvious sequence specificity[38,39]. This hinge region is the least conserved domain of HP1, which indicates that it could confer the selectivity of HP1 binding to specific cognate partners that might, for example, target the distinct HP1 isoforms to different locations. So, the properties of the variable hinge region might contribute not only to the basal nuclear localization of HP1 proteins, but also promote an increase in the concentration of HP1 proteins at discrete sites in the nucleus. This could account, at least in part, for the enrichment in HP1 proteins at centromeric regions.

The chromoshadow domain functions as a dimerization module and contains a vital consensus pentapeptide that is found in several nuclear proteins that interact with HP1 (REF. 40). Indeed, this domain of HP1α mediates interactions with either HP1β or HP1α itself[41]. Such self-association might contribute to heterochromatin compaction and possibly cohesion (clustering)[21]. In addition to self-association, HP1 interacting partners include the DNA methyltransferases Dnmt1 and Dnmt3a, which are involved in CpG methylation[42]. Whereas Dnmt3a is required for *de novo* DNA methylation[43], Dnmt1 seems primarily to maintain heritable methylation patterns during DNA replication[44]. In addition, the HP1 chromoshadow domain binds the large subunit p150 of chromatin-assembly factor-1 (CAF1)[45], a factor that is implicated in histone deposition during both DNA replication and repair[46]. Notably, CAF1 itself interacts with the DNA-replication-processivity clamp, proliferating cell nuclear antigen (PCNA), which provides a molecular link with both DNA replication and repair[46].

These properties of HP1 can therefore contribute to the stability of a domain that is enriched in HP1 in two principal ways. First, the number of HP1 interactors offers various possibilities for HP1-binding sites that, individually, might not have a strong affinity for HP1, but together could ensure the maintenance of a highly concentrated subregion. Second, all of the binding partners of HP1 that have already been discussed are proteins that are involved either in setting specific epigenetic marks on DNA or nucleosomes, or in promoting nucleosome assembly, and each of them can participate in the maintenance of pericentric heterochromatin.

Maintenance of pericentric heterochromatin. As the association of HP1 with pericentric heterochromatin is lost in *Suv39h* double-null primary mouse fibroblasts[28], the binding of HP1 to methylated H3-K9 was proposed to be crucial for the targeting of HP1 to pericentric heterochromatin[28,29,47]. It seems that trimethylated H3-K9 is enriched at pericentric heterochromatin[48,49]. However, although crucial, the recognition of methylated H3-K9 might not be sufficient by itself to ensure the stability of domains that are enriched in HP1. Considering that the affinity of HP1 for methylated amino-terminal histone-H3 peptide is rather low[50], several methylated H3-K9 sites might help to maintain high amounts of HP1 at a steady state in the pericentric heterochromatin, or, other distinct HP1-interaction sites could be involved. The H3-K9 modification is found at other loci, such as the inactive X chromosome in female cells, which are not enriched for HP1. Although HP1 is not excluded from these sites, it is clear that the H3-K9 modification is not sufficient for HP1 specificity[36,51,52]. This might result from a 'masking' mechanism that prevents direct access to the HP1-binding site, perhaps owing to other marks that are present, such as the *Xist* RNA mark on the inactive X chromosome[53]. Alternatively, a combinatorial system might be necessary for recognition, and therefore the

HISTONE-CODE HYPOTHESIS
A hypothesis that proposes that distinct histone modifications, on one or more tails, function sequentially, or in combination, to define a code that is translated by effector proteins with specific biological functions.

DOSAGE COMPENSATION
A process by which the expression of sex-linked genes is equalized in species in which males and females differ in the number of sex chromosomes. In *Drosophila melanogaster* dosage compensation is achieved by hypertranscription of the single male X chromosome.

H3-K9 modification, by itself, might not be sufficient for HP1 binding. The latter mechanism is consistent with observations indicating that other parameters are necessary for HP1 to be detected at pericentric heterochromatin. Indeed, evidence of the importance of the hypoacetylation of histones is provided by the fact that prolonged exposure of mammalian cells to a deacetylase inhibitor (which results in histone hyperacetylation) disrupts HP1 proteins that were initially concentrated at pericentric heterochromatin[6]. As the level of H3-K9 methylation is not concommitantly altered[36], both H3-K9 acetylation and methylation can occur on distinct H3 termini in pericentric heterochromatin. In addition, the importance of a combination of histone modifications is supported by the phenotype that is observed in cells in which an essential component of the Sin3–HDAC (suppressor-interacting–histone-deacetylase) corepressor complex has been inactivated[54].

Even more surprisingly, an RNA component is important, as the treatment of mouse cells with RNaseA disrupts the localization of HP1 at pericentric heterochromatin; this can be restored by simply adding back purified total or nuclear RNA[36]. A structural role can therefore be attributed to this RNA, which, in conjunction with the modification state of H3 (hypoacetylated and methylated at K9), participates in creating the specific higher-order structure that is typical of pericentric heterochromatin. How does this RNA participate to maintain the local concentration of HP1 at pericentric heterochromatin? One possibility is that the RNA-dependent architecture of heterochromatin might bring methylated H3-K9 amino-terminal ends together in a specific configuration that is required for the accumulation of HP1 in pericentric heterochromatin. Indeed, RNaseA treatment disrupts not only HP1 localization, but also the spatial ordering of methylated H3-K9, as detected by an antiserum that preferentially recognizes the spatial arrangement of H3-K9 methylation. Another possibility is that this RNA binds directly to the hinge region of HP1 and therefore promotes the architectural organization of the entire domain. So, it is interesting to note that transcripts corresponding to the major satellite repeats have been detected by either northern analysis or RT-PCR (PCR after reverse trancription of RNA)[55,56], but it is unclear whether they actually contribute to HP1 retention.

Taken together, these observations indicate that the local concentration of HP1 within pericentric heterochromatin depends on the following parameters: the presence of Suv39h, H3-K9 methylation, hypoacetylated histones and an RNA component — all of which are potential binding partners of HP1. The question, then, is how the many HP1 binding partners work together to achieve the functional organization of heterochromatin?

HP1 dynamics and inheritance during replication

Recent work on the dynamic properties of HP1 proteins within the cell nucleus might shed light on these issues. Heterochromatin has long been viewed as an inert, highly condensed and inaccessible domain, in which areas of HP1 concentration were thought to function as 'glue' that is bound in place without dynamic exchange. In contrast to this static view, experiments that were carried out using live-cell imaging and fluorescence recovery after photobleaching (FRAP) show that HP1 is not stably bound to pericentric heterochromatin, but is highly mobile and forms a stable compartment that is actively renewed[57,58]. Therefore, the dynamic properties of HP1 can take advantage of a multiplicity of low-affinity binding sites and could be crucial for the creation of an ever-changing, but overall stable, architectural framework, within which nuclear processes can take place.

DNA replication of heterochromatin presents several challenges, as the cell needs to replicate not only the DNA, but also the spatial organization of the whole domain. Furthermore, the passage of the replication fork is a disrupting event for nucleosomal organization[59], as well as for transcription complexes[60]. This disruption might actually be vital for fork progression, which could explain the presence of remodelling factors that might facilitate the process by remodelling the chromatin structure to allow the movement of the replication fork. Indeed, an imitation switch (ISWI)-containing chromatin-remodelling complex accumulates in pericentric heterochromatin during its replication[61], and another ISWI-containing complex is required for efficient replication of heterochromatin[62]. Other chromatin-remodelling proteins have been shown to bind HP1 (REF. 63,64), which suggests that, in addition, they have a role in the maintenance of heterochromatin.

Remarkably, during DNA replication, the overall concentration of HP1 is maintained at pericentric-heterochromatin regions[65], which indicates that either the nucleosomal destabilization that is generated by the replication fork is a local phenomenon that does not affect the stability of overall chromatin structure, or, that specific mechanisms are involved to ensure the maintenance of the domain. Here, we present a model that integrates the combination of HP1-binding partners, HP1 dynamic properties and chromatin assembly during DNA replication (FIG. 3), such that when one HP1-binding site is displaced, an alternative binding partner can be provided. The dynamic interaction properties of HP1 (FIG. 2) therefore enable us to propose a useful framework to explain how a stable structure — pericentric heterochromatin — can be propagated.

During replication, modified parental histones are displaced ahead of the replication fork and are randomly distributed between the two daughter strands[66]. Concomitantly, deposition of *de novo* synthesized histones provides the full complement of histones that are needed to ensure the proper assembly of the duplicated material. This latter step is mediated by CAF1, which is targeted to the replication fork through a direct association with PCNA[67,68]. In this scheme, we suggest that, whereas the passage of the replication fork is likely to abolish the association of HP1 with DNA, methylated H3-K9 and potentially RNA, the presence of CAF1 at the replication fork could provide HP1-binding sites to

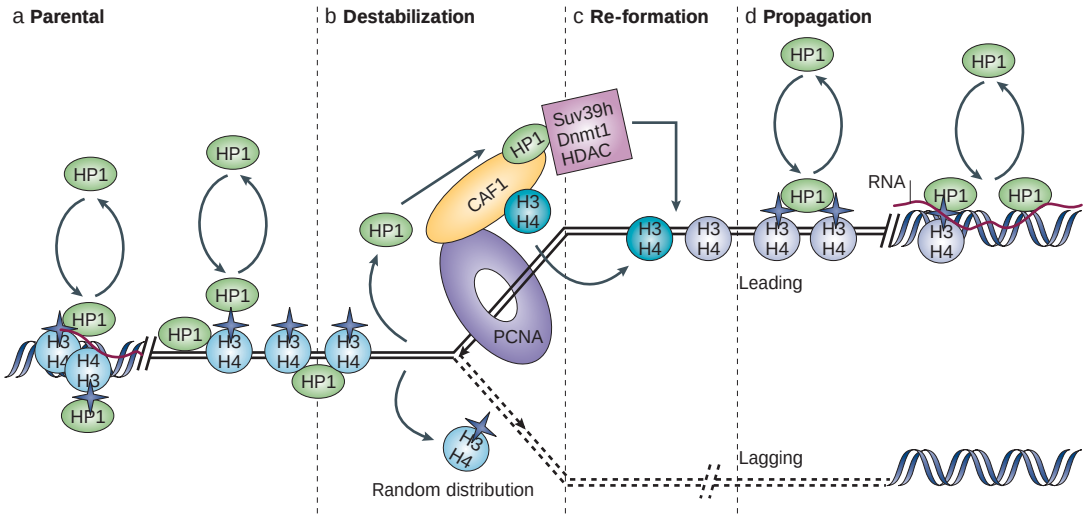


Figure 3 | **The ins and outs at the replication fork: a model for heterochromatin propagation.** For simplicity, only the leading strand, on which continuous DNA synthesis occurs, is shown in detail. During replication, the dynamics of histones involve: the random distribution between the two daughter strands of the parental histones (mid-blue circles); and concomitantly, the deposition of *de novo* synthesized histones acetylated on histone H4 (green-blue circles). Starting from parental heterochromatic DNA (**a**), on which heterochromatin protein-1 (HP1) is associated with numerous partners (such as methylated histone H3-K9 (methylation is represented by a blue star), DNA and RNA), the passage of the replication fork triggers the release of parental modified nucleosomal histones (methylated on histone H3) ahead of the fork, thereby leading to local destabilization and the loss of HP1 nucleosomal binding sites (**b**). New binding sites are subsequently created through the proliferating cell nuclear antigen (PCNA)-mediated recruitment of chromatin assembly factor-1 (CAF1), which assists *de novo* histone deposition in the 're-formation' step (**c**). The continual exchange between free and bound forms of HP1 ensures the plasticity of the domain. The CAF1-bound HP1 recruits the DNA methyltransferase Dnmt1 and its binding partner histone deacetylase (HDAC) to methylate DNA on CpG and deacetylate histones (pale-blue circles), respectively. CAF1-bound HP1 also recruits Suv39h to methylate histone H3 on K9. This latter modification, in turn, provides new HP1-binding sites to propagate the heterochromatin state ('propagation' step; **d**). Soon thereafter, newly synthesized DNA, in the form of chromatin bound to HP1, can associate with the 'structural RNA' that helps to ensure the architectural organization of the entire domain.

compensate for this. Once bound to CAF1, HP1 could, in turn, recruit the proteins that are involved in establishing epigenetic marks such as DNA methylation and histone modification (deacetylation and methylation). Newly synthesized histone H4 that is acetylated on residues K5 and K12 — a highly conserved pattern among eukaryotes[69] — is incorporated during replication and deacetylated shortly after deposition in heterochromatin[65]. So far, it remains unclear if, in mammalian cells, newly synthesized histone H3 is incorporated into heterochromatin with existing modifications. In our model, the methylation mark of histone H3-K9 would need to be imposed by a histone methyltransferase that is bound to HP1 to create new HP1-interacting sites and help to propagate the heterochromatin state. However, it is also possible to envisage that the methylation mark is already there in newly synthesized histone H3 and is therefore directly coupled to nucleosome assembly[70]. It should be noted that there are distinct histone-H3 variants in mammals and in *D. melanogaster*[71]. Notably, in the latter case, the deposition of the major histone-H3 variant is coupled to DNA synthesis during DNA replication, whereas histone H3.3 is deposited independently of DNA replication and marks active loci[72]. Therefore, it will be interesting to determine, in addition to the histone modifications, which histone-H3 variant is incorporated during heterochromatin replication and which mechanisms enable the perpetuation of the pre-existing state.

From this model, we suggest that, during the replication process, CAF1 functions as a chaperone for both newly synthesized histones and HP1 as previously proposed[45]. In this scheme, the PCNA–CAF1–HP1 complex could function as a platform that simultaneously contributes to both the maintenance and the duplication of the heterochromatic domain. However, the need for CAF1 specifically for heterochromatin duplication has yet to be demonstrated.

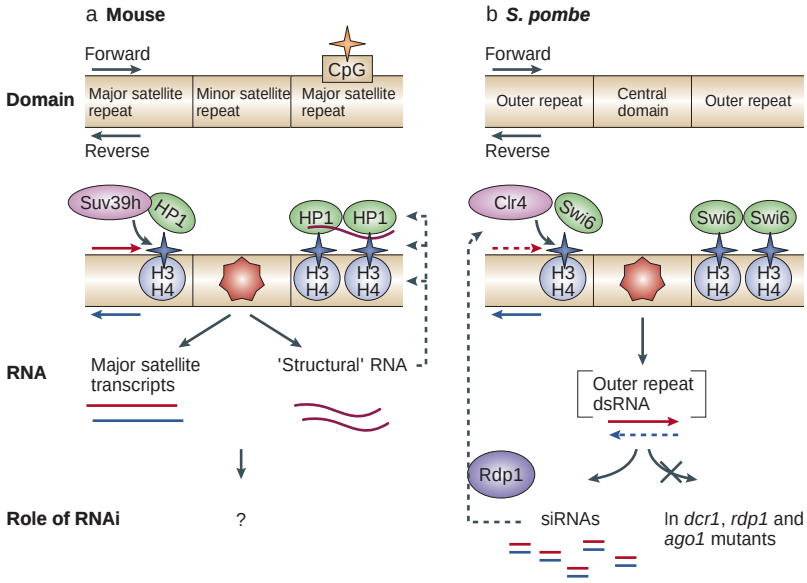
An unexpected role for RNAs

It is intriguing to envisage how RNA, the product of transcription, can participate in the organization of domains that are largely transcriptionally silent, particularly when the domains are thought to be condensed and inaccessible. However, in mouse cells, although pericentric heterochromatin was thought to be transcriptionally inactive, RNA transcripts that correspond to both strands of the centromeric major satellite repeats have been detected[55,56]. The dynamic properties of HP1, as discussed above, might enable the domain to be flexible enough to allow some transcription, which might then be quickly shut down, perhaps when the domain is made accessible during replication. What functions can be assigned to these non-coding RNAs?

Box 1 | Organization of pericentric heterochromatin in mouse cells and fission yeast

Domain structure

Regions of pericentric heterochromatin within centromeric regions are composed of numerous DNA repeats that are known as major satellite repeats in mouse cells and outer repeats in the fission yeast *Schizosaccharomyces pombe* (see figure). These repeats are heavily methylated in mouse cells (on CpG; DNA methylation is shown by an orange star) but not in *S. pombe*. Hypoacetylated histones and methylated histone H3-K9 (histone methylation is shown by a blue star) are present as well as the H3-K9 histone methyltransferase, which is known as Suv39h in mouse cells and Clr4 in *S. pombe*. H3-K9 modification provides binding sites for HP1 proteins in mouse cells and Swi6 in *S. pombe*. The centric region of the inner domain is represented by a red eight-pointed star in the figure.



RNA

In mouse cells, there are RNA transcripts of unknown function that are homologous to both strands of the major satellite repeat, as well as an RNA component ('structural' RNA) that is implicated in the maintenance of pericentric-heterochromatin organization. In *S. pombe* wild-type strains, transcripts from the outer repeats that are detected only in the reverse orientation (solid blue arrow) should be degraded post-transcriptionally by the RNA interference (RNAi) machinery. The level of transcription from outer-repeat DNA in the forward orientation is low (dotted red arrow). In mutant strains lacking Argonaute (Ago1), Dicer (Dcr1) or RNA-dependent RNA polymerase (Rdp1), which all affect the RNAi machinery, both forward and reverse transcripts remain stable.

Role of RNAi

In *S. pombe* wild-type strains, transient double-stranded RNA (dsRNA) molecules are processed into small interfering RNAs (siRNAs) by the RNAi machinery. These siRNAs promote methylation of H3-K9, allowing for the initiation of the heterochromatic state, thereby repressing transcription in the forward orientation. The continuous regeneration of dsRNA is ensured by priming of the reverse transcript by Rdp1, which is bound to the chromatin (dotted black arrow). How a comparable RNAi-dependent heterochromatin-formation process might occur in mouse cells is open to question.

The role of non-coding RNAs. The presence of non-coding RNAs that are associated with specific nuclear domains has recently created a lot of excitement[73–76]. For example, *roX1/2* RNA is associated with dosage compensation in *D. melanogaster*[77], *Xist* RNA is involved in X INACTIVATION in mammals[53], the production of double-stranded RNA (dsRNA) can cause transcriptional and post-transcriptional silencing in plants[78], and, even more striking, ribosomal-DNA transcription (or ribosomal RNA itself) is necessary to maintain nucleolar organization in the nucleus[79]. As discussed above, an unknown RNA component is required for the maintenance of mouse pericentric-heterochromatin organization, and is likely to participate directly or indirectly in the architectural organization[36]. Whether this RNA arises from local or distal transcription events, it should ultimately be incorporated into a nucleoprotein complex within heterochromatin to achieve its structural role.

The importance of RNA was highlighted in *S. pombe*, in which the RNA INTERFERENCE (RNAi) machinery is required for the formation of silent, condensed heterochromatin at centromeres[80] and at the mating-type region[81], and for centromere function[82,83]. Comparison of the common and distinct properties of the mouse and *S. pombe* systems; while taking evolutionary constraints into account, could reveal possible parallels in fundamental mechanisms.

Heterochromatin structure and organization. In terms of domain structure, the general organization of centromeric heterochromatin in mouse cells and in *S. pombe* is fairly similar (BOX 1). A central DNA domain, which is known as the minor satellite repeat in mouse cells and the central domain in *S. pombe*, is flanked on either side by regions of tandem DNA repeats, which are known as major satellite repeats in mouse cells and outer repeats in *S. pombe*. These pericentric regions are assembled into

X INACTIVATION
A process by which dosage compensation in mammals is achieved by the transcriptional silencing of one of the X chromosomes in XX females.

RNA INTERFERENCE
(RNAi). A post-transcriptional gene-silencing process in which double-stranded RNA triggers the degradation of homologous mRNA.

heterochromatin that is characterized in both organisms by the presence of methylated H3-K9, and by the binding of HP1 in mouse cells and its homologue Swi6 in *S. pombe*. The H3-K9 modification depends on the H3-K9 methyltransferase, known as Suv39h in mouse cells and Clr4 in *S. pombe*[47]. Importantly, the DNA (CpG) methylation that is found in mouse pericentric heterochromatin is not present in the *S. pombe* strains that have been studied so far. It should be noted that a DNA-methyltransferase-2-like protein in *S. pombe* seems to be enzymatically inactive owing to an amino-acid subsitution at a potential catalytic site[84].

The RNAi machinery in S. pombe. In *S. pombe*, the deletion of either ARGONAUTE, DICER or RNA-DEPENDENT RNA POLYMERASE (Rdp1), which are all components of the RNAi machinery, leads to the loss of H3-K9 methylation, binding of Swi6 and the silencing of transgenes that are inserted into centromeric heterochromatin[80]. In such mutants, overlapping forward and reverse centromeric transcripts could be detected. In a parallel study, small RNAs, which resembled Dicer cleavage products and had similarity to the centromeric outer repeats, were identified in wild-type strains[85]. On the basis of these findings, a model was proposed[80] in which centromeric outer repeats that are transcribed in both directions can create overlapping non-coding RNA that is then processed by the RNAi machinery (BOX 1). The resulting SMALL INTERFERING RNA (siRNA), in turn, somehow promotes the methylation of H3-K9 at the centromeric region, which allows the binding of Swi6 and the initiation of the silenced heterochromatin state. This requires a functional H3-K9 methyltransferase, Clr4 (REF. 47). Rdp1 that is physically associated with centromeric heterochromatin ensures the continuous regeneration of dsRNAs and siRNAs to maintain silent heterochromatin, a reaction that is favoured in the context of tandem repeats, as found in pericentric regions[86]. Recent studies in *S. pombe* show that the expression of synthetic hairpin RNAs is sufficient to silence corresponding homologous loci in *trans* and to induce the assembly of silent chromatin with all of the hallmarks of centromeric heterochromatin, such as H3-K9 methylation and Swi6 binding. This process requires both the RNAi machinery and Clr4 (REF. 87). Normally expressed genes can therefore be targeted for heterochromatin formation in this way. How the siRNAs are actually able to recognize the appropriate region to induce silencing remains unclear, but pairing between siRNA and either homologous DNA sequences or nascent RNA transcripts at the target locus is a probable explanation[34,78]. This hypothesis is supported by the recent discovery of the RITS (RNA-induced initiation of transcriptional gene silencing) complex that is required for heterochromatin assembly in *S. pombe*. This complex contains small RNAs that are homologous to centromeric regions and are necessary for the localization of RITS to heterochromatic domains[88].

A role for RNAi in mammals? It is tempting to propose a similar connection between the RNAi machinery and heterochromatin formation and maintenance in complex organisms such as mammals. However, the situation in mammals might be functionally more difficult to unravel. Whereas the genome of *S. pombe* contains only one gene that encodes each of the heterochromatin proteins (such as Swi6 and the proteins that are involved in the RNAi machinery), in mammals there are numerous diverse genes that encode HP1, H3-K9 methyltransferase and some of the RNAi components such as Argonaute[89]. Furthermore, components of the RNAi machinery that are found in *S. pombe* might not necessarily be present in mammals. For example, genome-wide sequence analysis has not found any sequence homologues of Rdp1 in mammals or *D. melanogaster*[90]. However, considering the key role of Rdp1 in ensuring the connection of chromatin with histone modification[80], mammalian cells might have evolved alternative pathways. The fact that DNA methylation is found in mammalian cells but not in *S. pombe* adds a further layer of complexity, as DNA methylation is thought to contribute to the stability of heterochromatin in higher eukaryotes[91]. In plants and fungi, feedback mechanisms between DNA methylation (distinct from the CpG that is found in mammals) and histone methylation were found[92–94], indicating that the two processes might function together to maintain the silent chromatin state.

Such species differences raise the issue of whether or not a comparable RNAi-dependent heterochromatin-formation process occurs in mammals. One possibility that could integrate most parameters is to envisage that a sequential mechanism would take place over time. During development, RNAi could be important to initiate the process. Once the structure is established, maintenance of the process would depend on another mechanism that functions by self propagation, for example, during replication as discussed above. In this respect, it is interesting to point out that embryonic stem cells contain a much higher level of Dicer proteins than differentiated cells[95],which could contribute to an initiating event. The DNA methylation would find its importance at later stages to ensure self-propagation and maintenance through subsequent cellular divisions.

This model does not exclude the possibility that, in *S. pombe*, in addition to the transcripts that are involved in the RNAi pathways for the initiation of heterochromatin formation, there might be other structural RNAs that maintain heterochromatic regions. Future work on these exciting issues will help us to better understand how the basic principles may have evolved to establish domains with comparable functional properties.

Implications/future directions

The maintenance of pericentric heterochromatin is essential to ensure the proper segregation of chromosomes and the regulation of gene expression. Besides several epigenetic marks, the prominent mark of this region is the enrichment in HP1. Its multipartite organization enables it to bind to numerous nuclear proteins. Some of these proteins are potentially involved in maintaining the stability of the higher-order structure of pericentric heterochromatin. A local concentration of

ARGONAUTE
A family of proteins that are characterized by the presence of two homology domains (PAZ and PIWI) that are essential for the production or function of small interfering RNAs. So far, their biochemical function has not been characterized.

DICER
An RNaseIII-type nuclease that is required for the processing of double-stranded-RNA precursors into small interfering RNAs.

RNA-DEPENDENT RNA POLYMERASE
(Rdp1). An unusual RNA polymerase that uses small interfering RNAs as primers to generate long double-stranded RNA, which in turn can be processed by Dicer.

SMALL INTERFERING RNA
(siRNA). During RNA interference, these non-coding RNAs (around 22 nucleotides long) are derived from the processing of long double-stranded RNA. They direct the destruction of mRNA targets that have the same sequence.

HP1 is therefore proposed to be dependent on the presence of numerous binding partners that, individually, have a relatively low affinity. The nature of HP1 interaction, combined with the presence of various different partners within the domain, means that is could possibly exchange partners and contribute to a structure that can be highly dynamic. These various properties of HP1 can be integrated into a model to explain how a stable structure, pericentric heterochromatin, can be propagated. These data set the stage for future work that is based on the idea of self-organization of nuclear domains[96]. By virtue of many transient interactions, HP1 proteins in pericentric heterochromatin can illustrate the principles of using self-organization to determine an architectural domain, and this could apply more widely to other macroscopic subnuclear compartments. These recent findings have changed our views of previous models of heterochromatin organization. Heterochromatin seems to be a much more dynamic entity than we initially imagined, in which transcription can occur and participate in setting up the heterochromatic state.

The implication of RNA and the RNAi machinery in organizing nuclear domains is probably just the beginning of all the possible non-anticipated roles of RNA in the nucleus. Whether the RNAi machinery contributes to the pericentric-heterochromatin structural organization in mammals should be addressed. Although many pieces of the puzzle are still missing, exciting working models can now be experimentally tested, and this will enable us to establish links between molecular and macromolecular entities. A challenge for the future will be to understand how epigenetic properties can be faithfully propagated throughout multiple rounds of cell division. How, during replication, the combinatorial loading of all the marks and the incorporation of the specific histone variants that have been associated with particular chromatin states[72] is achieved is a question that must be answered. In this respect, it is interesting to note that specialized assembly pathways and dedicated histone chaperones can be involved[97]. How these can be coordinated with HP1 maintenance has now to be considered, to support or discard the working model proposed here.

1. Flemming, W. *Zell substanz, Kern und Zelltheilung*, (Vogel, Leipzig, 1882).
2. Heitz, E. Das heterochromatin der Moose. *Jb. Wiss. Bot.* **69**, 728 (1928).
3. Sullivan, B. A., Blower, M. D. & Karpen, G. H. Determining centromere identity: cyclical stories and forking paths. *Nature Rev. Genet*. **2**, 584–596 (2001).
4. Dillon, N. & Festenstein, R. Unravelling heterochromatin: competition between positive and negative factors regulates accessibility. *Trends Genet.* **18**, 252–258 (2002).
5. Vissel, B. & Choo, K. H. Mouse major (γ) satellite DNA is highly conserved and organized into extremely long tandem arrays: implications for recombination between nonhomologous chromosomes. *Genomics* **5**, 407–414 (1989).
6. Taddei, A., Maison, C., Roche, D. & Almouzni, G. Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. *Nature Cell Biol.* **3**, 114–120 (2001).
7. Peters, A. H. *et al*. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).
8. Ekwall, K., Olsson, T., Turner, B. M., Cranston, G. & Allshire, R. C. Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. *Cell* **91**, 1021–1032 (1997).
9. Francastel, C., Walters, M. C., Groudine, M. & Martin, D. I. A functional enhancer suppresses silencing of a transgene and prevents its localization close to centromeric heterochromatin. *Cell* **99**, 259–269 (1999).
10. Brown, K. E., Baxter, J., Graf, D., Merkenschlager, M. & Fisher, A. G. Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. *Mol. Cell* **3**, 207–217 (1999).
11. Henikoff, S. Position-effect variegation after 60 years. *Trends Genet.* **6**, 422–426 (1990).
12. Bird, A. DNA methylation patterns and epigenetic memory. *Genes Dev.* **16**, 6–21 (2002).
13. Jeppesen, P., Mitchell, A., Turner, B. & Perry, P. Antibodies to defined histone epitopes reveal variations in chromatin conformation and underacetylation of centric heterochromatin in human metaphase chromosomes. *Chromosoma* **101**, 322–332 (1992).
14. James, T. C. & Elgin, S. C. Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. *Mol. Cell. Biol.* **6**, 3862–3872 (1986).
15. James, T. C. *et al*. Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of *Drosophila*. *Eur. J. Cell Biol.* **50**, 170–180 (1989).
16. Eissenberg, J. C. *et al*. Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **87**, 9923–9927 (1990).
17. Singh, P. B. *et al*. A sequence motif found in a *Drosophila* heterochromatin protein is conserved in animals and plants. *Nucleic Acids Res.* **19**, 789–794 (1991).
18. Minc, E., Courvalin, J. C. & Buendia, B. HP1γ associates with euchromatin and heterochromatin in mammalian nuclei and chromosomes. *Cytogenet. Cell Genet.* **90**, 279–284 (2000).
19. Nielsen, A. L. *et al*. Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell* **7**, 729–739 (2001).
20. Gilbert, N. *et al*. Formation of facultative heterochromatin in the absence of HP1. *EMBO J.* **22**, 5540–5550 (2003).
21. Jones, D. O., Cowell, I. G. & Singh, P. B. Mammalian chromodomain proteins: their role in genome organisation and expression. *Bioessays* **22**, 124–137 (2000).
22. Ball, L. J. *et al*. Structure of the chromatin binding (chromo) domain from mouse modifier protein 1. *EMBO J.* **16**, 2473–2481 (1997).
23. Nielsen, P. R. *et al*. Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. *Nature* **416**, 103–107 (2002).
24. Brasher, S. V. *et al*. The structure of mouse HP1 suggests a unique mode of single peptide recognition by the shadow chromo domain dimer. *EMBO J.* **19**, 1587–1597 (2000).
25. Cowieson, N. P., Partridge, J. F., Allshire, R. C. & McLaughlin, P. J. Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Curr. Biol.* **10**, 517–525 (2000).
26. Fischle, W. *et al*. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* **17**, 1870–1881 (2003).
27. Li, Y., Kirschmann, D. A. & Wallrath, L. L. Does heterochromatin protein 1 always follow code? *Proc. Natl Acad. Sci. USA* **99**, 16462–16469 (2002).
28. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116–120 (2001).
29. Bannister, A. J. *et al*. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120–124 (2001).
30. Strahl, B. D. & Allis, C. D. The language of covalent histone modifications. *Nature* **403**, 41–45 (2000).
31. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
32. Turner, B. M. Cellular memory and the histone code. *Cell* **111**, 285–291 (2002).
33. Aagaard, L. *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.* **18**, 1923–1938 (1999).
34. Grewal, S. I. & Moazed, D. Heterochromatin and epigenetic control of gene expression. *Science* **301**, 798–802 (2003).
35. Akhtar, A., Zink, D. & Becker, P. B. Chromodomains are protein–RNA interaction modules. *Nature* **407**, 405–409 (2000).
36. Maison, C. *et al*. Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. *Nature Genet.* **30**, 329–334 (2002).
This paper shows that a RNA component is required for the maintenance of the mouse pericentric-heterochromatin organization.
37. Muchardt, C. *et al*. Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1α. *EMBO Rep.* **3**, 975–981 (2002).
38. Sugimoto, K., Yamada, T., Muro, Y. & Himeno, M. Human homolog of *Drosophila* heterochromatin-associated protein 1 (HP1) is a DNA-binding protein which possesses a DNA-binding motif with weak similarity to that of human centromere protein C (CENP-C). *J. Biochem. (Tokyo)* **120**, 153–159 (1996).
39. Meehan, R. R., Kao, C. F. & Pennings, S. HP1 binding to native chromatin *in vitro* is determined by the hinge region and not by the chromodomain. *EMBO J.* **22**, 3164–3174 (2003).
40. Smothers, J. F. & Henikoff, S. The HP1 chromo shadow domain binds a consensus peptide pentamer. *Curr. Biol.* **10**, 27–30 (2000).
41. Le Douarin, B. *et al*. A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors. *EMBO J.* **15**, 6701–6715 (1996).
42. Fuks, F., Hurd, P. J., Deplus, R. & Kouzarides, T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res.* **31**, 2305–2312 (2003).
43. Okano, M., Bell, D. W., Haber, D. A. & Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257 (1999).
44. Robertson, K. D. DNA methylation and chromatin — unraveling the tangled web. *Oncogene* **21**, 5361–5379 (2002).
45. Murzina, N., Verreault, A., Laue, E. & Stillman, B. Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol. Cell* **4**, 529–540 (1999).
46. Mello, J. A. & Almouzni, G. The ins and outs of nucleosome assembly. *Curr. Opin. Genet. Dev.* **11**, 136–141 (2001).
47. Nakayama, J., Rice, J. C., Strahl, B. D., Allis, C. D. & Grewal, S. I. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* **292**, 110–113 (2001).
48. Rice, J. C. *et al*. Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol. Cell* **12**, 1591–1598 (2003).

49. Peters, A. H. *et al*. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* **12**, 1577–1589 (2003).
50. Jacobs, S. A. & Khorasanizadeh, S. Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. *Science* **295**, 2080–2083 (2002).
51. Peters, A. H. *et al*. Histone H3 lysine 9 methylation is an epigenetic imprint of facultative heterochromatin. *Nature Genet.* **30**, 77–80 (2002).
52. Chadwick, B. P. & Willard, H. F. Chromatin of the Barr body: histone and non-histone proteins associated with or excluded from the inactive X chromosome. *Hum. Mol. Genet.* **12**, 2167–2178 (2003).
53. Avner, P. & Heard, E. X-chromosome inactivation: counting, choice and initiation. *Nature Rev. Genet.* **2**, 59–67 (2001).
54. David, G., Turner, G. M., Yao, Y., Protopopov, A. & DePinho, R. A. mSin3-associated protein, mSds3, is essential for pericentric heterochromatin formation and chromosome segregation in mammalian cells. *Genes Dev.* **17**, 2396–2405 (2003).
55. Rudert, F., Bronner, S., Garnier, J. M. & Dolle, P. Transcripts from opposite strands of γ satellite DNA are differentially expressed during mouse development. *Mamm. Genome* **6**, 76–83 (1995).
56. Lehnertz, B. *et al*. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.* **13**, 1192–1200 (2003).
57. Cheutin, T. *et al*. Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* **299**, 721–725 (2003).
58. Festenstein, R. *et al*. Modulation of heterochromatin protein 1 dynamics in primary mammalian cells. *Science* **299**, 719–721 (2003).
References 57 and 58 demonstrate that the heterochromatin protein HP1 is highly dynamic within stable heterochromatin domains.
59. Gruss, C., Wu, J., Koller, T. & Sogo, J. M. Disruption of the nucleosomes at the replication fork. *EMBO J.* **12**, 4533–4545 (1993).
60. Wolffe, A. P. & Brown, D. D. DNA replication *in vitro* erases a *Xenopus* 5S RNA gene transcription complex. *Cell* **47**, 217–227 (1986).
61. Bozhenok, L., Wade, P. A. & Varga-Weisz, P. WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO J.* **21**, 2231–2241 (2002).
62. Collins, N. *et al*. An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. *Nature Genet.* **32**, 627–632 (2002).
63. McDowell, T. L. *et al*. Localization of a putative transcriptional regulator (ATRX) at pericentromeric heterochromatin and the short arms of acrocentric chromosomes. *Proc. Natl Acad. Sci. USA* **96**, 13983–13988 (1999).
64. Nielsen, A. L. *et al*. Selective interaction between the chromatin-remodeling factor BRG1 and the heterochromatin-associated protein HP1α. *EMBO J.* **21**, 5797–5806 (2002).
65. Taddei, A., Roche, D., Sibarita, J., Turner, B. & Almouzni, G. Duplication and maintenance of heterochromatin domains. *J. Cell Biol.* **147**, 1153–1166 (1999).
66. Sogo, J. M. & Laskey, R. A. in *Chromatin Structure and Gene Expression* (ed. Elgin, S. C. R.) 49–71 (Oxford Univ. Press, New York, 1995).
67. Shibahara, K. I. & Stillman, B. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* **96**, 575–585 (1999).
68. Moggs, J. G. *et al*. A CAF-1/PCNA mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.* **20**, 1206–1218 (2000).
69. Sobel, R. E., Cook, R. G., Perry, C. A., Annunziato, A. T. & Allis, C. D. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl Acad. Sci. USA* **92**, 1237–1241 (1995).
70. Vermaak, D., Ahmad, K. & Henikoff, S. Maintenance of chromatin states: an open-and-shut case. *Curr. Opin. Cell Biol.* **15**, 266–274 (2003).
71. Ahmad, K. & Henikoff, S. Epigenetic consequences of nucleosome dynamics. *Cell* **111**, 281–284 (2002).
72. Ahmad, K. & Henikoff, S. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191–1200 (2002).
73. Eddy, S. R. Non-coding RNA genes and the modern RNA world. *Nature Rev. Genet.* **2**, 919–929 (2001).
74. Storz, G. An expanding universe of noncoding RNAs. *Science* **296**, 1260–1263 (2002).
75. Zamore, P. D. Ancient pathways programmed by small RNAs. *Science* **296**, 1265–1269 (2002).
76. Finnegan, E. J. & Matzke, M. A. The small RNA world. *J. Cell Sci.* **116**, 4689–4693 (2003).
77. Park, Y. & Kuroda, M. I. Epigenetic aspects of X-chromosome dosage compensation. *Science* **293**, 1083–1085 (2001).
78. Matzke, M., Matzke, A. J. & Kooter, J. M. RNA: guiding gene silencing. *Science* **293**, 1080–1083 (2001).
79. Verheggen, C., Le Panse, S., Almouzni, G. & Hernandez-Verdun, D. Maintenance of nucleolar machineries and pre-rRNAs in remnant nucleolus of erythrocyte nuclei and remodeling in *Xenopus* egg extracts. *Exp. Cell Res.* **269**, 23–34 (2001).
80. Volpe, T. A. *et al*. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297**, 1833–1837 (2002).
81. Hall, I. M. *et al*. Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232–2237 (2002).
82. Hall, I. M., Noma, K. & Grewal, S. I. RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc. Natl Acad. Sci. USA* **100**, 193–198 (2003).
83. Volpe, T. *et al*. RNA interference is required for normal centromere function in fission yeast. *Chromosome Res.* **11**, 137–146 (2003).
References 81 and 83 demonstrate links between the RNA interference pathway and heterochromatin silencing in fission yeast.
84. Ramsahoye, B. H. *et al*. Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc. Natl Acad. Sci. USA* **97**, 5237–5242 (2000).
85. Reinhart, B. J. & Bartel, D. P. Small RNAs correspond to centromere heterochromatic repeats. *Science* **297**, 1831 (2002).
86. Martienssen, R. A. Maintenance of heterochromatin by RNA interference of tandem repeats. *Nature Genet.* **35**, 213–214 (2003).
87. Schramke, V. & Allshire, R. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science* **301**, 1069–1074 (2003).
88. Verdel, A. *et al*. RNAi-mediated targeting of heterochromatin by the RITS complex. *Science* **303**, 672–676 (2004).
89. Carmell, M. A., Xuan, Z., Zhang, M. Q. & Hannon, G. J. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. *Genes Dev.* **16**, 2733–2742 (2002).
90. Stein, P., Svoboda, P., Anger, M. & Schultz, R. M. RNAi: mammalian oocytes do it without RNA-dependent RNA polymerase. *RNA* **9**, 187–192 (2003).
91. Xu, G. L. *et al*. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402**, 187–191 (1999).
92. Tamaru, H. & Selker, E. U. A histone H3 methyltransferase controls DNA methylation in *Neurospora crassa*. *Nature* **414**, 277–283 (2001).
93. Jackson, J. P., Lindroth, A. M., Cao, X. & Jacobsen, S. E. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416**, 556–560 (2002).
94. Soppe, W. J. *et al*. DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. *EMBO J.* **21**, 6549–6559 (2002).
95. Billy, E., Brondani, V., Zhang, H., Muller, U. & Filipowicz, W. Specific interference with gene expression induced by long, double-stranded RNA in mouse embryonal teratocarcinoma cell lines. *Proc. Natl Acad. Sci. USA* **98**, 14428–14433 (2001).
96. Misteli, T. The concept of self-organization in cellular architecture. *J. Cell Biol.* **155**, 181–185 (2001).
97. Tagami, H., Ray-Gallet, D., Almouzni, G. & Nakatani, Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent on DNA synthesis. *Cell* **116**, 51–61 (2004).
This paper describes how histones H3.1 and H3.3 are deposited into chromatin through distinct pathways.

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Competing interests statement

The authors declare that they have no competing finacial interests.

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