CHROMATIN DYNAMICS

Epigenetic inheritance during the cell cycle

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Abstract | Studies that concern the mechanism of DNA replication have provided a major framework for understanding genetic transmission through multiple cell cycles. Recent work has begun to gain insight into possible means to ensure the stable transmission of information beyond just DNA, and has led to the concept of epigenetic inheritance. Considering chromatin-based information, key candidates have arisen as epigenetic marks, including DNA and histone modifications, histone variants, non-histone chromatin proteins, nuclear RNA as well as higher-order chromatin organization. Understanding the dynamics and stability of these marks through the cell cycle is crucial in maintaining a given chromatin state.

Epigenetics
This term was coined by Waddington in 1942 to describe how genes of a genotype bring about a phenotype. Current definitions of epigenetics include the study of heritable changes in gene function that occur without alterations to the DNA sequence.

Centromere
A region of a chromosome that is defined by the presence of a centromere-specific histone H3 variant (CenH3) and that functions as a platform for kinetochore assembly during mitosis.

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The definition of epigenetics has received much attention, as attested by the number of recent publications[1–6]. When originally coined by Waddington in 1942, the term epigenetics defined the causal mechanisms by which the genes of a genotype bring about a phenotype[7]. On revisiting this definition in 1987, Holliday applied the term epigenetic to situations in which changes in DNA methylation result in changes in gene activity[8]. Today, the most widely accepted definition — which we adopt in this Review — designates epigenetics as the study of heritable changes in genome function that occur without alterations to the DNA sequence[1]. This definition implies that particular states that define cell identity are attained by heritable instructions — the epigenetic marks that determine whether, when and how particular genetic information will be read. The initial setting up of these epigenetic marks represents an establishment phase. Here, we discuss epigenetic inheritance as the means to ensure the transmission of epigenetic marks, once they are established, from mother to daughter cell and potentially from generation to generation. Therefore, epigenetic information provides a form of memory that is necessary for the maintenance of genome function, including both the differential gene expression patterns of a given cell lineage (encompassing, for example, the maintenance of a cell identity after differentiation, position-effect variegation in *Drosophila melanogaster*, dosage compensation and imprinting in mammals) and the propagation of essential architectural features, such as telomeres and centromeres, that are required for cell viability or proliferation status. Any unscheduled compromise at these levels might lead to disease.

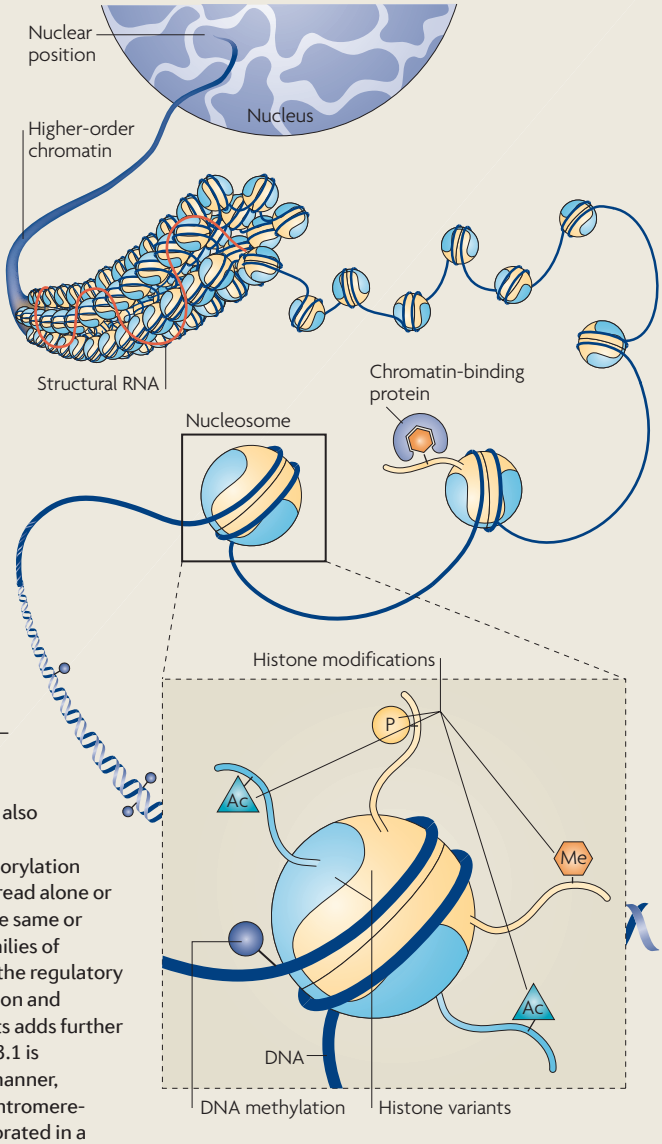
Recent research has highlighted DNA methylation as a bona fide epigenetic mark, and chromatin organization has emerged as a source of major candidates for carriers of information superimposed on that encoded by DNA itself (BOX 1). In line with genetic information, epigenetic marks must be heritable to qualify as true epigenetic information. Furthermore, in contrast to genetic information, which is meant to be highly stable, epigenetic information reveals a certain level of plasticity and is inherently reversible. Therefore, one needs to understand how a particular chromatin state that is associated with a particular cell type can survive through multiple cell divisions and, more specifically, how it can face the dramatic perturbation that occurs during the passage of the replication fork in S phase. Depending on the nature of the epigenetic mark, different strategies to restore or maintain epigenetic states operate, either immediately following the disruptive event (that is, in a replication-coupled manner) or in a manner that can be separated in time from the disruptive event.

The centromere is an attractive model to discuss the concept of epigenetic inheritance during the cell cycle (BOX 2). It presents a paradigm for an epigenetically defined locus, because its functionality is not ensured by the underlying DNA sequence but rather by its particular chromatin organization[9]. Once established, centromere organization and function have to be stably maintained through multiple cell divisions to ensure proper chromosome segregation. Given the essential role of centromeres, the proper inheritance of epigenetic marks, including the higher-order organization, which define centromeres, must endure chromatin disruption during the passage of the replication fork or the repair

Box 1 | **Candidate players for epigenetic inheritance**

Epigenetic inheritance refers to the transmission of information beyond the DNA sequence during cell division and from one generation to the next[1,3]. Inheritance of epigenetic information is crucial for maintaining differential gene expression patterns in differentiation, development and disease. Candidates for key players in epigenetic inheritance that are situated of different levels of chromatin include DNA and histone modifications, histone variants, non-histone chromatin proteins that bind directly to DNA or to histone modifications, nuclear RNA and higher-order organization, as well as positional information. We need to distinguish between marks that reflect short-term instructions and can quickly revert in response to a signal (for example, heat shock or damage) and those that are long-term instructions. These long-term instructions might be inherited independently of the initial trigger, might qualify as epigenetic marks and could contribute to cellular memory[2].

DNA wraps around a histone octamer that is composed of one $(H3–H4)_2$ tetramer capped by two H2A–H2B dimers. Together with the linker histone H1, this forms the nucleosome — the basic building block of chromatin (see the figure). DNA itself is covalently modified by methylation of cytosine residues. Histones are also post-translationally modified (for example, by methylation (Me), acetylation (Ac) and phosphorylation (P)), and each mark constitutes a signal that is read alone or in combination with other modifications on the same or neighbouring histones as a 'histone code'. Families of methyl- or histone-binding proteins decipher the regulatory information that is encoded by DNA methylation and histone marks. The presence of histone variants adds further complexity. Whereas the replicative variant H3.1 is incorporated in a DNA synthesis-dependent manner, replacement variants, such as H3.3 and the centromere-specific histone H3 variant CenH3, are incorporated in a DNA synthesis-independent manner and result in nucleosomes with atypical stability. Nucleosomal chains fold into higher-order chromatin structures that are potentially organized with non-coding RNA components. The position of a particular chromosomal domain in the nucleus constitutes an additional level of instructions for gene expression.



of damaged DNA. The basic rules that can be learnt from the maintenance of a well-defined domain, such as the centromere, might further our understanding of the general principles that underlie the inheritance of epigenetic states.

The actual nature and diversity of histone modifications and modifiers[10], and histone variants[11], have been covered elsewhere, as have the challenges posed to chromatin during replication and repair[12,13]. Here, we discuss the sophisticated mechanisms that have evolved in order to facilitate the inheritance of epigenetic marks not only at the replication fork, but also at other stages of the cell cycle. This Review provides an overview of our current knowledge concerning the inheritance of DNA methylation, histone modifications and histone variants that is either coupled or not coupled to DNA replication. We discuss the maintenance of heterochromatin using the example of centromeres and show, by means of reprogramming events that occur during development, the reversibility of epigenetic marks and their dynamics.

Heterochromatin
A chromatin region that remains condensed throughout the cell cycle and that is characterized by a specific chromatin signature.

Reprogramming
The induced reversal of an epigenetic state, resulting in an altered cellular identity.

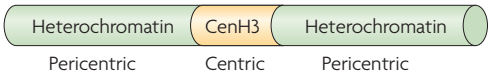
Inheritance at the replication fork

In each cell cycle, the integrity of genetic and epigenetic information is challenged during DNA replication. When DNA replicates, chromatin undergoes a wave of disruption and subsequent restoration in the wake of the passage of the replication fork. Whereas lineage preservation requires the faithful maintenance of epigenetic marks, DNA replication also presents a window

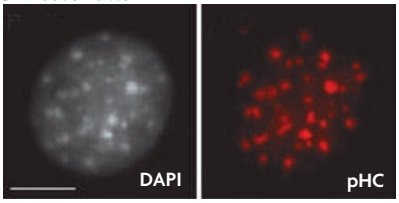
Box 2 | **Heterochromatin at centromeres**

Centromeres are key chromosomal elements that are responsible for correct chromosome segregation at each cell division[94]. Whereas in budding yeast the incorporation of the centromere-specific histone H3 variant CenH3 is determined by a particular DNA sequence, such a sequence requirement has been lost during evolution[9]. At most centromeres, rapidly evolving repetitive sequences are found and centromere function is determined by chromatin organization and the presence of CenH3. Therefore, centromeres are a paradigm for an epigenetically defined domain. They consist of a central domain, called the inner centromere or centric heterochromatin, which is at the basis of kinetochore formation and where CenH3 is incorporated (see the figure, part **a**). The adjacent pericentric heterochromatin (pHC) contributes to centromere function by ensuring sister chromatid cohesion[103,104,124]. Pericentric heterochromatin remains condensed throughout the cell cycle and individual pericentromeres come together into large clusters called chromocentres[124], as shown by DNA fluorescence *in situ* hybridization (FISH) for pericentric satellite repeats in mouse embryonic fibroblasts (see the figure, part **b**). At the molecular level, pericentric heterochromatin is characterized by extensive DNA methylation and specific histone methylation marks, such as dimethylated and trimethylated H3K9 (H3K9me2 and H3K9me3, respectively), that are bound by heterochromatin protein 1 (HP1; see the figure, part **c**). There are three HP1 proteins in mammals: HP1α, HP1β and HP1γ (also known as CBX5, CBX1 and CBX3, respectively). RNA interference (RNAi) contributes to heterochromatin integrity in fission yeast and plants[172]; however, a direct connection in flies and mammalian cells is so far lacking. Not every epigenetic mark is present at pericentric heterochromatin in all model organisms. Scale bar, 5 μm. DAPI, 4′,6-diamidino-2-phenylindole.

a



b Mouse nuclei



c Centromere characteristics in different organisms

Organism	DNA sequence requirement	Centromere-specific H3 variant	DNA methylation	H3K9 methylation	HP1	RNAi pathway
Saccharomyces cerevisiae	Yes	Cse4	No	No	No	No
Schizosaccharomyces pombe	No	Cnp1	No	Yes	Yes	Yes
Drosophila melanogaster	No	CID	No	Yes	Yes	Yes?
Arabidopsis thaliana	No	HTR12	Yes	Yes	No	Yes
Mammals	No	CENP-A	Yes	Yes	Yes	Unknown

of opportunity for changes in epigenetic states to occur during differentiation and development. Thus, refined mechanisms have evolved to ensure stability through the concerted transmission of genetic and epigenetic information at the replication fork, and to ensure plasticity that allows the desired switches during development. Understanding how to deal with this dual requirement is a fascinating issue into which we have begun to gain insight.

Inheritance of DNA methylation during replication. Since the first proposal that genetic information is replicated in a semi-conservative manner[14], much has been learned about the enzymes and machinery at work during replication[15]. However, it is only beginning to emerge how, at the replication fork, the inheritance of genetic and epigenetic information can be coupled and how components of the DNA replication machinery potentially crosstalk with all of the aspects of inheritance beyond the DNA sequence.

DNA replication proceeds in an asymmetric manner with continuous synthesis on the leading strand and discontinuous synthesis on the lagging strand (FIG. 1a,b). This synthesis is catalysed by specialized DNA polymerases on each strand[16]. DNA polymerases are assisted by the DNA processivity factor proliferating cell nuclear antigen (PCNA)[17], which is loaded onto both strands. Thus, PCNA provides an important link between the two strands, and folding of the two strands in space might further ensure the coupling of replication mechanisms on both leading and lagging strand[18] (FIG. 1a). When considering epigenetic marks, in addition to duplicating DNA, it is important to evaluate how DNA methylation, histone deposition and histone marks are connected to the replication machinery. In addition to its role in DNA synthesis, PCNA might also link DNA synthesis and the inheritance of epigenetic marks[19], as suggested by the early observation that particular mutations in PCNA suppress position-effect variegation in *D. melanogaster*[20]. Furthermore, PCNA interacts with many chromatin-assembly and chromatin-modifying factors[12,13,19,21,22] (FIG. 1c; see below). In addition to PCNA, other factors are likely to contribute to the crosstalk between the inheritance of genetic and epigenetic information. Indeed, the minichromosome maintenance (MCM) complex, which is the putative replicative helicase, interacts with the histone chaperone anti-silencing function 1 (ASF1; see below)[23], which is proposed to coordinate histone flow on parental and daughter strands.

Similar to the semi-conservative inheritance of DNA sequences, patterns of symmetrical DNA methylation at CpG (cytosine followed by guanine) sites are transmitted

Histone chaperone
A factor that associates with histones and stimulates a reaction that involves histone transfer without being part of the final product.

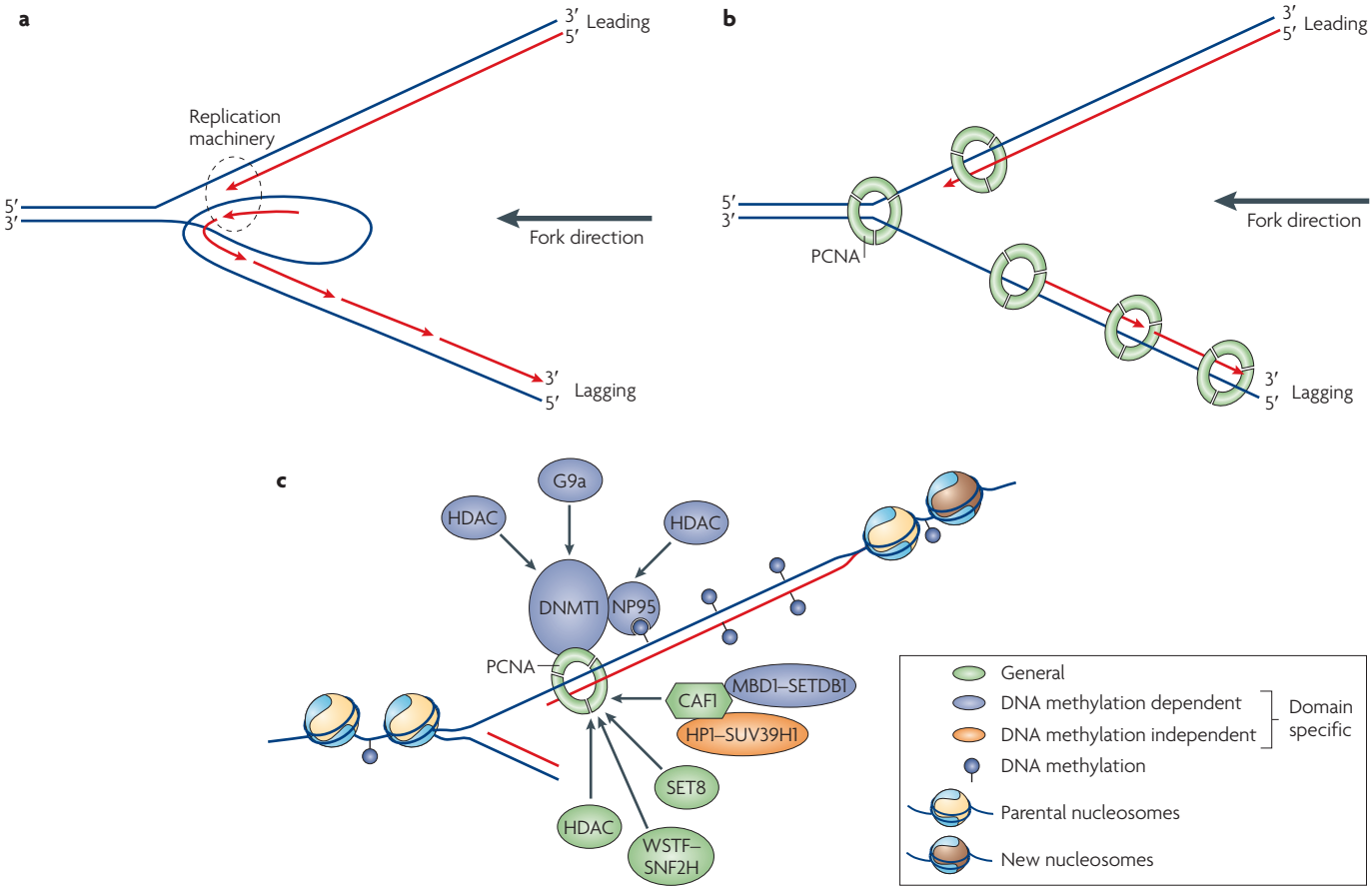


Figure 1 | **Asymmetric DNA replication and coupling of inheritance of DNA and histone marks. a** | An intrinsic strand bias at DNA replication. DNA replication occurs in the 5′ to 3′ direction. One strand is replicated as the leading strand and the other as the lagging strand[18]. **b** | Proliferating cell nuclear antigen (PCNA) molecules associate with the 3′ end of newly synthesized DNA. This results in the loading of PCNA on to the two strands. **c** | Maintenance of DNA and specific histone modifications at the replication fork. Homotrimeric PCNA recruits general factors that function at all forks, such as histone modifiers (histone deacetylases (HDACs) and the Lys methyltransferase SET8 (also known as KMT5A, PR-SET7 and SETD8))[73–75], chromatin remodellers (Williams syndrome transcription factor (WSTF)–SNF2H (also known as SMARCA5))[76] and chromatin assembly factor 1 (CAF1; also known as CHAF1)[21]. Depending on the presence of DNA methylation, PCNA together with NP95 (also called UHRF1 and ICBP90) recruits DNA methyltransferase 1 (DNMT1), which methylates hemimethylated CpG sites on daughter strands[26,32,33]. Certain histone modifiers use the DNA methylation machinery as a template — for example, HDAC activity is recruited by DNMT1 and NP95 (REFS 81,82), and DNMT1 interacts with the Lys methyltransferase G9a (also known as KMT1C)[83]. In DNA methylation-rich regions, CAF1 forms a complex with methyl CpG-binding protein 1 (MBD1) and the Lys methyltransferase SETDB1 (also known as KMT1E), thereby coupling histone deposition with histone methylation[79,80]. CAF1 also contributes to the maintenance of heterochromatin protein 1 (HP1) in a DNA-methylation-independent process[128,130]. HP1, in turn, interacts with the histone methyltransferase SUV39H1 (also known as KMT1A)[68].

with high fidelity. The maintenance of DNA methylation at the fork is ensured by DNA methyltransferase 1 (DNMT1), owing to its affinity for hemimethylated DNA *in vitro*[24,25] and its interaction with PCNA[26]. However, the mechanism by which methylation maintenance is ensured in a faithful manner was unclear, as DNMT1 also shows *de novo* methylation activity[27] and its ability to bind PCNA is not absolutely required for DNA methylation maintenance[28,29]. Recent evidence now suggests that the SET- and RING-associated (SRA)-domain-containing proteins variant in methylation 1 (VIM1) in *Arabidopsis thaliana* and NP95 (also called UHRF1 and ICBP90) in mammals constitute an additional mechanistic link between hemimethylated DNA and DNMT1 (REFS 30–33). NP95 binds preferentially to hemimethylated DNA[34–36], interacts with DNMT1 and is required for its localization to replicating heterochromatic regions[32] (FIG. 1c). Indeed, deletion of NP95 results in methylation defects[33] that resemble those that are observed following the loss of DNMT1 (REF. 37), which suggests that NP95 has a dominant role in tethering maintenance methyltransferase activity to newly replicated DNA. The maintenance of DNA methylation further requires the ATP-dependent chromatin-remodelling factor decreased DNA methylation 1 (DDM1) in *A. thaliana*[38,39] and LSH (also known as HELLS) in mice[40], which have been suggested to provide access of the methylation machinery to newly replicated DNA[38].

DNA methyltransferase
An enzyme that transfers methyl groups from *S*-adenosylmethionine to specific adenines or cytosines in DNA.

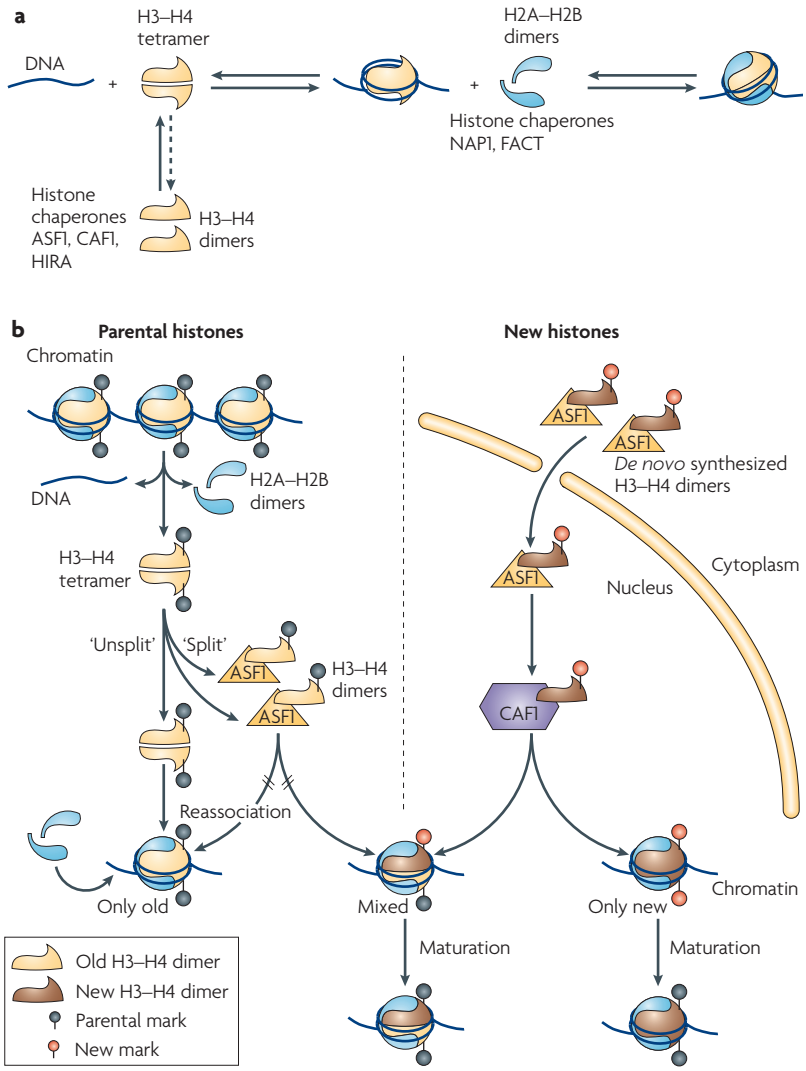


Figure 2 | **Nucleosome dynamics and mixing of parental and new H3–H4 dimers.** **a** | The incorporation of histone $(H3–H4)_2$ tetramers onto DNA, followed by the addition of two histone H2A–H2B dimers to form a nucleosome core particle. Prior to deposition, H3–H4 and H2A–H2B exist as dimers that are complexed to specific histone chaperones. **b** | On chromatin disruption at replication, parental H3–H4 tetramers with histone marks can either be preserved (unsplit) or broken up into dimers (split), potentially by interacting with the chaperone anti-silencing function 1 (ASF1)[62,63]. Nucleosomes with only old H3–H4 are formed when unsplit parental tetramers are transferred directly onto daughter strands or when two parental H3–H4 dimers reassociate. Newly synthesized H3–H4 dimers with their typical marks are complexed with the chaperones ASF1 and chromatin assembly factor 1 (CAF1; also known as CHAF1)[59]. Nucleosomes might be formed on the daughter strands from one parental and one new H3–H4 dimer (indicated as mixed) or exclusively from two new H3–H4 dimers (indicated as only new). Nucleosomes that contain mixed and new histones undergo maturation after formation. FACT, facilitates chromatin transcription; HIRA, Hir-related protein A; NAP1, nucleosome assembly protein 1.

DNA methylation patterns can be reproduced faithfully after the passage of the replication fork by taking advantage of a combination of factors: semi-conservative replication, which gives rise to hemimethylated DNA; the recognition of the hemimethylated daughter strand by NP95; and the association of DNMT1 with the replication machinery. These mechanisms ensure a stable propagation of DNA methylation patterns and reinforce the view that DNA methylation is a prototype of a bona fide epigenetic mark. Although we have learnt about the maintenance mechanisms that ensure the stable propagation of marks, it will also be important to consider mechanisms that enable the removal of these marks to fully comprehend the dynamic behaviour of DNA methylation, as suggested by recent reports[41–43].

Inheritance of histones and their modifications? DNA and its methylation marks are replicated using semi-conservative mechanisms of inheritance, in which information is copied from a template[44]. Passage of the replication fork also disrupts parental nucleosomes that carry post-translational modifications. In order to be heritable and therefore to qualify as epigenetic marks, these histones and their modifications must be correctly reassembled behind the fork[13]. However, an obvious template for nucleosome reassembly is lacking. Given that outside of S phase the exchange of the replicative histone H3 variant H3.1 and histone H4 is minimal compared with the rapid exchange of H2A and H2B[45,46], H3 and H4, along with their associated marks, have arisen as likely candidates to transmit information from one cell cycle to the next. Therefore, to avoid the loss of information that is encoded in histone modifications, proper coordination is required between the recycling of parental H3–H4 dimers with their histone marks, along with the incorporation of newly synthesized histones[13].

Nucleosome assembly involves the deposition of one $(H3–H4)_2$ tetramer, which can exist in an intermediate H3–H4 dimeric form, onto DNA, followed by the deposition of two H2A–H2B dimers[47] (FIG. 2a). Histone chaperones have key roles as histone acceptors and donors that assist in the disruption and reassembly of nucleosomes. They control histone provision locally and exhibit specificity for particular histones or even a specific histone variant[48]. Importantly, the H3.1–H4 chaperone chromatin assembly factor 1 (CAF1; also known as CHAF1) is recruited to the replication fork through an interaction with PCNA along with other histone modifiers, such as histone deacetylases (HDACs) and Lys methyltransferases[19,21] (see below). CAF1 is composed of three subunits — p150, p60 and p48 — that coordinate nucleosome assembly during DNA replication[49,50] or at sites of DNA repair[22,51] by facilitating the deposition of newly synthesized H3.1–H4 (REF. 52).

Another H3–H4 chaperone, ASF1, interacts directly with the CAF1 p60 subunit[53] and functions synergistically with CAF1 in DNA synthesis-dependent chromatin assembly by acting as a donor of newly synthesized histones. Furthermore, ASF1 is directly linked to the replication fork machinery through interactions with components of the putative replicative helicase[23]. Downregulation of ASF1 slows down S-phase progression and impairs DNA unwinding because of defects in histone dynamics[23]. The newly synthesized histones that are associated with chaperones, such as CAF1 and ASF1, carry the evolutionarily conserved combination of the K5 and K12 acetylation marks on H4 (REFS 54,55), which are associated with the deposition of new histones and are removed during chromatin maturation. In budding yeast, new H3 is acetylated at residue K56 (H3K56ac), which

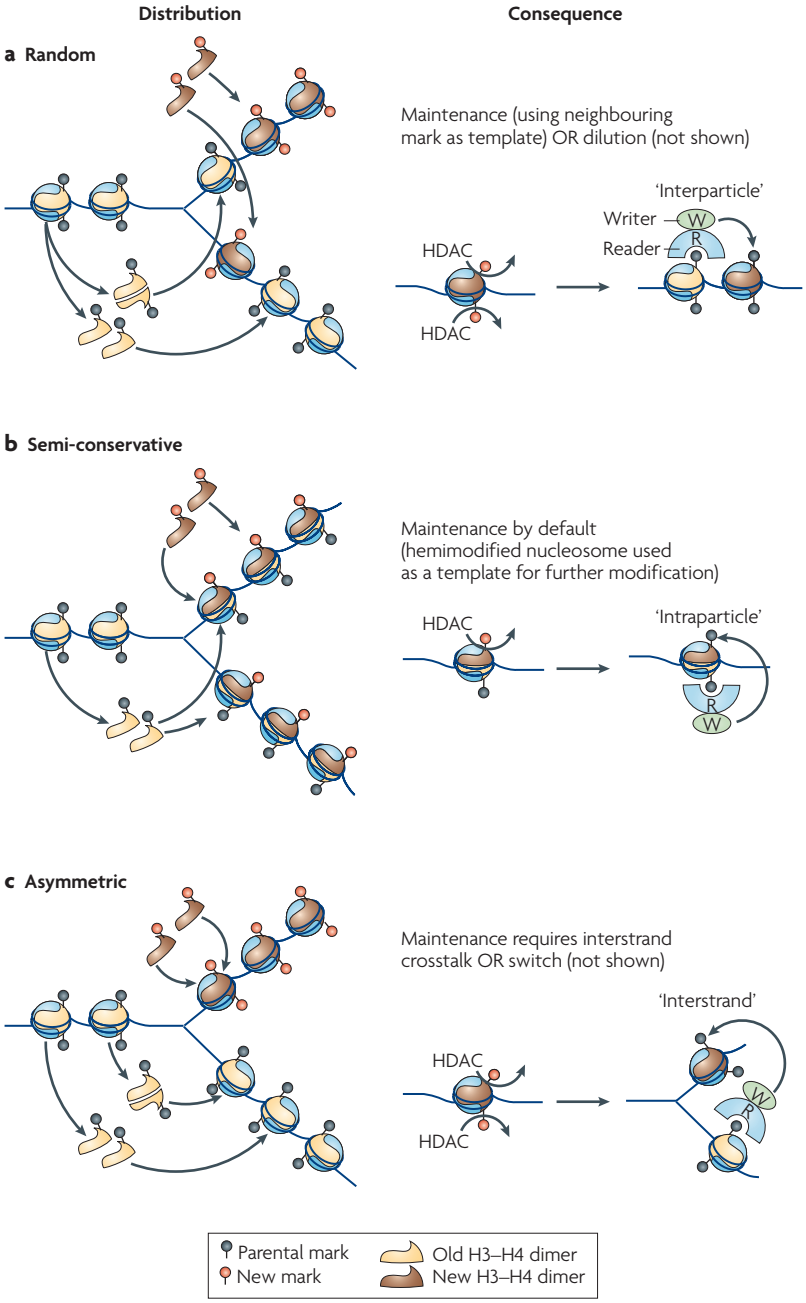


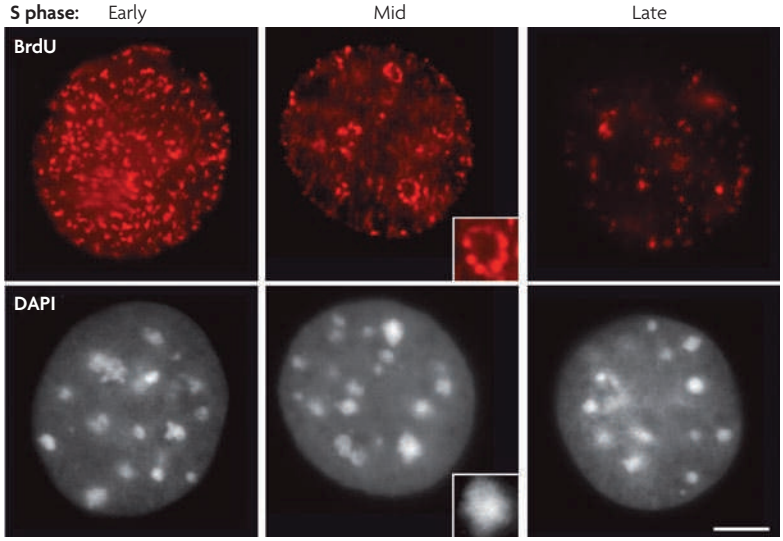
Figure 3 | **Fate of old and new H3–H4 dimers and their marks at the fork.** Three possibilities for the distribution of parental histones are presented. In each case, the parental mark is recognized by a chromatin-binding protein, or reader protein, that in turn recruits a chromatin modifier, or writer protein. **a** | Random histone distribution. Parental histone H3 and H4 with marks (unsplit or reassociated dimers) are distributed randomly onto daughter strands and chromatin density is restored by the deposition of new H3–H4 dimers. To avoid the dilution of histone marks, active maintenance requires first a deacetylation step, which involves a histone deacetylase (HDAC), followed by histone modification that is guided by neighbouring parental nucleosomes (an interparticle process). **b** | Semi-conservative histone distribution. Parental dimers with marks segregate evenly onto each daughter strand and nucleosomes are completed by the deposition of new H3–H4 dimers. After deacetylation, 'hemimodified' nucleosomes provide a template for the transmission of parental marks to newly deposited H3–H4 dimers (an intraparticle process). **c** | Asymmetric histone distribution. Parental H3–H4 dimers with marks are redistributed onto daughter strands in an asymmetric manner. This is possibly dictated by the intrinsic strand bias that is introduced during DNA replication, and induces a switch from one chromatin state to another. The maintenance of histone modifications requires interstrand crosstalk.

regulates the nucleosome assembly that is dependent on CAF1 and the yeast-specific histone chaperone Rtt106 (REFS 56,57). Whereas the presence of the H3K56ac mark has been reported in humans[58], its abundance seems limited and its association with new histone deposition is not documented. Furthermore, homologues of Rtt106 and the Lys acetyltransferase Rtt109 (also known as Kat11), which acts on H3K56, have yet to be identified in humans. So, whether H3K56ac or an unidentified modification have similar roles in mammals remains to be investigated.

Notably, newly synthesized histones H3 and H4 are present as dimers in pre-deposition complexes with histone chaperones[59] (FIG. 2a). Although this principle is clearly established for newly synthesized histones, the fate of parental H3–H4 histone dimers that are thought to be deposited as tetramers on the daughter strands might also have to be reconsidered. The fact that histones H3 and H4 exist as stable tetramers in solution in the absence of DNA[60] argues against the existence of parental H3–H4 dimers. However, structural data now show that the association of ASF1, and potentially also p48 and p55, with histones is incompatible with a tetrameric structure[61–65]. In addition, the fact that some histones carrying parental marks can be detected in association with ASF1 under conditions in which the helicase and the polymerase are uncoupled[23] supports the hypothesis that ASF1 is involved in tetramer splitting and that it functions as an acceptor of recycled parental dimers. Therefore, it is indeed possible that parental tetramers (with their own marks) are split and redistribute onto daughter strands as dimers. This affects histone dynamics at the fork and might produce either mixed tetramers that comprise parental and new dimers (FIG. 2b), or nucleosomes that comprise only old histones if parental dimers reassociate. This second scenario requires either that the old dimers are held in close contact or away from the new ones, or that some recognition event ensures that the correct old dimers are brought back together in the same particle. The spatial organization of DNA at the fork might facilitate these mechanisms (FIG. 1a).

If modifications on the new histones are guided by modifications of parental histones, the way in which parental histones are distributed to the daughter strands will determine the degree of conservation of histone marks. Current models suggest that the distribution of both parental and newly synthesized histones onto daughter strands occurs in a random fashion (FIG. 3a). To avoid the dilution of histone marks, the maintenance of modifications could be achieved by using a neighbouring histone as a template. A possible mechanism could be envisaged in which the parental mark is recognized by a chromatin-binding protein, or reader protein[66], that in turn recruits a chromatin modifier, or writer protein. This has been suggested for the self-reinforcing loop in the maintenance of heterochromatin protein 1 (HP1) at pericentric heterochromatin[67–70] (see below). Such a mechanism probably operates in repetitive regions in which long arrays of nucleosomes carry the same marks, but cannot apply to regions in which particular

Box 3 | **Spatial and temporal regulation of replication**



In the nucleus, distinct chromatin domains occupy different compartments and replicate at different times, with the classic example of heterochromatin usually replicating late and euchromatin early[173]. Such a temporal and spatial replication programme highlights the capacity of the cell to distinguish one domain from another. Characteristic patterns of replication can be visualized in cells that are labelled with nucleotide analogues at different times during S phase[174,175]. In mice, at least three S-phase patterns that occupy different subnuclear compartments can be distinguished[128] — early, mid and late S phase — as shown by pulse labelling with a nucleotide analogue (see the figure).

Note that when pericentric heterochromatin replicates in mid S phase, pericentric domains remain visible by 4′,6-diamidino-2-phenylindole (DAPI) staining, and bromodeoxyuridine (BrdU) incorporation is detected at the periphery of the domain, thereby revealing a specific organization. Replication timing patterns are also particular to the differentiation state of a cell[176]. Such a spatial and temporal organization could be exploited to self-maintain or propagate domains by facilitating the packaging of DNA into different types of chromatin during S phase, depending on when and where it is replicated[173,177]. This could be achieved by using local concentrations of specific factors[178] and/or by using specialized replication mechanisms. Proteins that are targeted to early or late replication forks could be instrumental in revealing how these features can translate into molecular terms to provide specificity. Histone deacetylase 2 (HDAC2)[82], methyl CpG-binding protein 2 (MBD2)–MBD3 (REF. 179) and Williams syndrome transcription factor (WSTF)–SNF2H[180] are examples of factors that are reported to be specific to late replication foci. A current challenge is to understand how spatial and temporal organization of replication is established and to solve the long-standing issue of the initiation of replication origins in mammals. Scale bar, 5 μm.

marks are restricted to only one or two nucleosomes. A similar self-maintaining process has now been proposed for the maintenance of the repressive methylation mark H3K27me3 (H3 trimethylated on residue K27) during replication, in which polycomb repressive complex 2 (PRC2) — which is responsible for setting the H3K27me3 mark — binds to its own methylation site[71]. It will be important to determine when the parental marks are actually imposed after the passage of the replication fork to evaluate how tightly the inheritance is coupled to replication.

Split parental tetramers could also distribute in a semi-conservative manner (FIG. 3b). As in the hemimethylated DNA scenario above, 'hemimodified' nucleosomes provide a template that instructs the appropriate choice of modification to impose on newly deposited H3–H4 histone dimers. A third possibility, which might occur at certain times and in certain chromatin domains, is that parental and new histones segregate asymmetrically[72]. This might be dictated by the intrinsic strand bias that is introduced at DNA replication, as discussed above (FIGS 1b,3c). This might help to induce a switch by providing a blank template to allow a change in cell fate on one of the two daughter strands. To faithfully copy information from parental to new nucleosomes, interstrand crosstalk would be required. Although evidence for this kind of mechanism is currently lacking, it is possible to envisage a folding in space that brings the two daughter strands into close proximity.

In summary, several models that are not necessarily mutually exclusive have been proposed to describe how new and recycled histones are incorporated and modified. It will be important to assess these models in different contexts (for example, different cell types and particular subdomains of the nucleus) in order to evaluate the effect of histone dynamics at the replication fork on the stability and plasticity of an epigenetic state.

Connecting inheritance of DNA and histone marks

In the reader–writer model for the inheritance of histone marks (FIG. 3), marks on neighbouring parental nucleosomes serve as a template for modifications of newly incorporated histones. This maturation step might take place at later stages in the cell cycle. However, marks could also be imposed in a replication-coupled manner and might be coordinated with the timing of domain replication (BOX 3). For replication-coupled maintenance, two situations can be considered: first, common factors at all replication forks can affect marking; and second, domain-specific factors are modulated by the local chromatin environment and by pre-existing marks, such as DNA methylation (FIG. 1c).

PCNA on all replication forks can function as a landing pad for different chromatin modifiers[17]. PCNA recruits HDACs[73] and the Lys methyltransferase SET8 (also known as KMT5A, PR-SET7 and SETD8), which is implicated in monomethylation of H4K20 (REFS 74,75) as well as chromatin remodelling activity[76]. PCNA, together with CAF1, remains on replicated DNA for ~20 min[77,78]. During this time window, newly replicated chromatin undergoes modifications, including the removal of acetylation marks on residues K5 and K12 of newly incorporated histone H4 (REFS 54,55).

At DNA methylation-rich regions, pre-existing methylation and its associated maintenance machinery could guide the placement of histone modifications. In those regions, methyl CpG-binding protein 1 (MBD1), which is found in a complex with the Lys methyltransferase SETDB1 (also known as KMT1E)[79], can interact with CAF1 during replication[80], which suggests that there is a connection between histone deposition and the setting of modifications. Reported interactions of the DNA methyltransferase enzyme DNMT1 with the histone-modifying enzymes HDAC1 (REF. 81), HDAC2 (REF. 82) and the Lys methyltransferase G9a (also known as KMT1C)[83], might ensure a coordination between the imposition of marks on DNA and histones.

Histone H3 variant
A replicative histone H3 variant is expressed and incorporated during DNA replication (for example, H3.1 and H3.2), whereas a replacement variant is expressed throughout the cell cycle and is incorporated in a DNA-synthesis-independent manner (for example, H3.3 and the centromere-specific histone H3 variant CenH3).

Particular features that are created at the time of DNA replication in a particular domain might also be exploited. NP95 has affinity for both hemimethylated DNA and histones[34–36,84], and specifically interacts with peptides that are methylated at H3K9 *in vitro*[85] by potentially reading histone marks. In addition, NP95 was found in a complex with HDACs and G9a[31,32]. Therefore, as well as binding to hemimethylated DNA, NP95 could interpret the histone environment, thereby creating a feedback mechanism that involves the mutual reinforcement of histone and DNA methylation marks. In this situation, histone marks would influence the inheritance of DNA methylation. Further chromatin-binding proteins or chromatin modifiers with dual affinity for both DNA methylation and a particular histone modification are likely to be identified.

The examples above show how histone and DNA methylation in a repressive domain could be maintained at the replication fork. However, how active chromatin marks are propagated is less clear. Recently, transmission of an active state through nuclear transfer in *Xenopus laevis* has been reported, and it has been proposed that the replacement histone variant H3.3 is required for epigenetic memory[86]. To evaluate this hypothesis, it is necessary to better understand the mechanisms that involve replication-independent histone exchange processes and the replacement of histone variants.

Inheritance of histone variants outside S phase

Histone variants can mark a particular chromatin state: H3.3 is enriched at active regions, whereas the unique incorporation of the centromere-specific histone H3 variant CenH3 (CENP-A in humans) specifies the site of centromere identity. Together with the replicative variants H3.1 and H3.2, the replacement variants H3.3 and CENP-A constitute the major histone H3 isotypes that are known in mammals[87]. During S phase, H3.1 and H3.2 are exclusively incorporated, whereas the deposition of replacement variants, such as H3.3 or CENP-A, occurs outside S phase[11,88]. Thus, the histone variants H3.3 and CENP-A have emerged as candidates for key players of epigenetic information that can be transmitted in a replication-independent manner.

Inheritance of H3.3. H3.3 is associated with transcriptionally active regions and is enriched in active histone marks[55,89,90]. Furthermore, nucleosomes that contain H3.3 seem to be less stable than those that contain H3.1 (REF. 91). The extent to which this depends on the differential modification status of the nucleosomes[55], the presence of other variants, such as H2A.Z[92], or inherent differences in their structural properties remains to be established. Regardless, *in vivo*, these properties suggest that H3.3 nucleosomes are more dynamic or amenable to displacement during transcription. Given that replication leads to a concomitant deposition of H3.1, the density of H3.3-containing nucleosomes is reduced. As the mixing of H3.1 and H3.3 in the same nucleosome has not been observed[55,59], a semi-conservative mechanism at the fork is unlikely for H3.3 inheritance (FIG. 3b).

It is possible that the dilution of H3.3 and its marks by one-half after one cell cycle might not affect the transcriptional readout of a region. Thus, sustained active gene expression, combined with modifications on parental H3.3, might recruit factors that modify newly incorporated H3.1 with the appropriate marks, and H3.3 incorporation might be stimulated. Consistent with this hypothesis, arrays of nucleosomes that contain both H3.3 and H3.1 nucleosomes have been observed, and analysis of histone modifications in this context show that, when adjacent to H3.3 nucleosomes, H3.1 nucleosomes accumulate active marks[55]. However, dilution of H3.3 over a number of generations might be reconciled by the replication-independent incorporation of H3.3 that is promoted by chaperones, such as Hir-related protein A (HIRA), following transcription[59,93] (FIG. 4a).

Inheritance of CENP-A. The histone H3 variant CENP-A marks the site of centromere identity[94,95]. The association of CENP-A with centromeres is extremely stable, as shown by quantitative fluorescence recovery after photobleaching (FRAP) analysis, and it remains associated through cell division[96]. Although the exact mechanism of CENP-A deposition at centromeres remains enigmatic, it is a replication-independent process, as is the deposition of H3.3 (REF. 97). CENP-A deposition was first proposed to occur in G2 phase, because CENP-A assembly can take place in the presence of the DNA replication inhibitor aphidicolin, and *CENPA* mRNA and the CENP-A protein peak in G2 phase[97]. Recent evidence in mammalian cells now suggests that the loading of new CENP-A onto centromeres is restricted to a discrete cell cycle window in late telophase–early G1 phase[98], but the mechanism and the specific chaperone that facilitate CENP-A deposition remain to be deciphered.

Centromeric DNA is replicated during S phase, in which parental CENP-A nucleosomes are distributed to daughter strands[97,98]. Therefore, chromatin at the centromeres contains one-half of the complement of CENP-A nucleosomes after the completion of S phase and during subsequent G2 and M phases. To reconcile the deficit in CENP-A molecules, current models predict that during replication, either H3.1-containing nucleosomes are temporarily placed at centromeres, or, alternatively, nucleosome 'gaps' are created that are filled later in the cell cycle[99] (FIG. 4b).

Recent studies suggest that CENP-A nucleosomes are unusual and that these peculiarities might provide a means of marking this region of the chromosome as unique. For example, in budding yeast, a specialized Cse4 (*Saccharomyces cerevisiae* CenH3)-containing nucleosome has been proposed to exist in a form in which histones H2A and H2B are replaced by the non-histone protein suppressor of chromosome missegregation 3 (Scm3)[100]. In *D. melanogaster*, a 'hemisome' that consists of one molecule each of CenH3, H4, H2A and H2B has been described[101]. Additional evidence suggests that, like H3.3 nucleosomes, CENP-A nucleosomes are easier to disassemble *in vitro* than canonical nucleosomes[102]. One might speculate that 'unusual' CENP-A-containing nucleosomes represent centromeric chromatin in an intermediate

Histone deacetylase
An enzyme that removes acetyl groups from histones.

Lys methyltransferase
An enzyme that catalyses the addition of a methyl group to specific Lys residues in histones and other non-histone proteins.

Lys acetyltransferase
An enzyme that catalyses the addition of an acetyl group to specific Lys residues in histones and other non-histone proteins.

Heterochromatin protein 1
(HP1). A chromodomain-containing protein that binds to methylated K9 on histone H3 and is associated with heterochromatin in fission yeast (Swi6), mammals (HP1) and *Drosophila melanogaster* (HP1).

Pericentric heterochromatin
A heterochromatic region adjacent to chromatin containing the centromere-specific histone H3 variant CenH3, and which is considered to be typical constitutive heterochromatin.

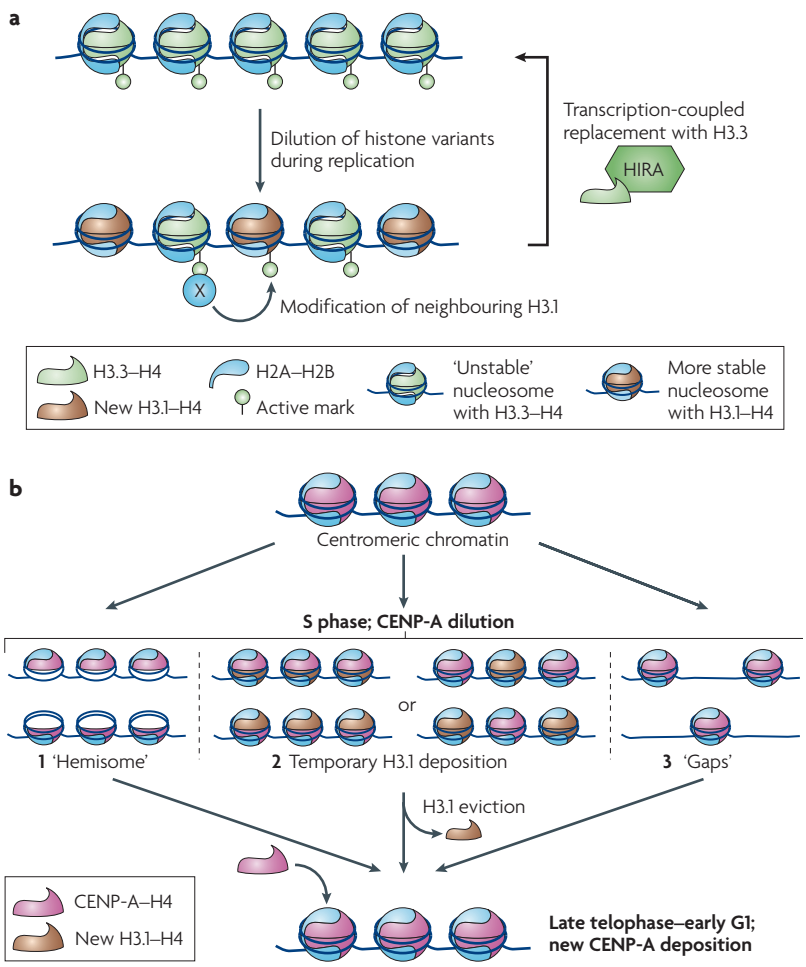


Figure 4 | **Inheritance of histone H3 variants outside of S phase.** **a** | Transcriptionally active domains are enriched in nucleosomes that contain histone H3.3, which have more dynamic conformations and are enriched in active marks[55,89–91]. Deposition of the histone variant H3.1 during DNA replication results in the dilution of H3.3. Active marks on H3.3 might recruit factors that facilitate the modification of neighbouring H3.1 to ensure the inheritance of an active state in a dominant fashion. Loss of H3.3 might be counterbalanced by the transcription-dependent incorporation of H3.3 promoted by histone chaperones, such as Hir-related protein A (HIRA), that function in chromatin assembly independently of DNA synthesis[59,89,93]. **b** | Incorporation of the centromere-specific histone H3 variant CenH3 (CENP-A in humans) at centromeres is another DNA-synthesis-independent histone-deposition process[97]. Replication of centromeric DNA in S phase dilutes CENP-A, resulting in three possible scenarios. First, parental CENP-A is equally distributed to daughter strands as a dimer, possibly creating hemisomes. Second, parental CENP-A is distributed onto daughter strands (as either tetramers or dimers) and H3.1 is temporarily deposited at centromeres, resulting in asymmetric or random distribution. Third, parental CENP-A is randomly distributed to daughter strands (as either tetramers or two dimers) and nucleosome 'gaps' are created[99,101]. Later in the cell cycle, during late telophase–early G1 phase, newly synthesized CENP-A is deposited at centromeres[98], possibly by specific deposition factors. Eviction of temporary H3.1 from centromeres might precede the deposition of new CENP-A.

state that contains one-half of the amount of CENP-A, before it is fully replenished with new CENP-A molecules later in the cell cycle. Although the incorporation of replacement variants H3.3 or CENP-A is not directly dependent on DNA replication, the distribution of parental histones at the fork could potentially pre-determine how and when H3.1 can be replaced at later stages. In this respect, examining the distribution of particular histone variants at particular domains throughout the cell cycle might prove to be highly informative.

Challenges of heterochromatin maintenance

Pericentric heterochromatin domains contribute to correct chromosome segregation and must be maintained throughout the cell cycle. During mitosis and S phase, the particular molecular marks that characterize pericentric heterochromatin and its higher-order organization (BOX 2) are challenged. In different organisms, such as fission yeast and mice, diverse mechanisms have evolved that ensure heterochromatin maintenance.

Fission yeast. Fission yeast spends most of its lifetime in G2 phase, during which pericentric repeats are organized into nucleosomes that are enriched in dimethylated H3K9 (H3K9me2), to which the HP1 homologue Swi6 is bound. Swi6 recruits the evolutionarily conserved ring-shaped protein complex cohesin, which maintains sister chromatid cohesion[103,104]. As cells enter mitosis, histone H3 becomes phosphorylated on residue S10, which results in reduced Swi6 binding and facilitates chromosome segregation[105–107]. Centromeres undergo replication even before cytokinesis is completed[108]. The dilution of repressive histone marks and further Swi6 delocalization as a consequence of DNA replication are thought to allow access to the RNA polymerase II machinery, and the bidirectional transcription of pericentromeric repeats occurs in this discrete cell cycle window of early S phase[106,107] (FIG. 5a).

Indeed, a careful analysis of transcript levels during the cell cycle reveals a correlation between the timings of replication and transcription, as the forward transcripts that have a transcription start site closer to the replication origin accumulate first[107]. The transcripts are processed into small interfering RNAs (siRNAs) that accumulate transiently in S phase[107]. RNA interference (RNAi)-dependent and RNAi-independent mechanisms then direct Lys methyltransferase (Clr4; also known as Kmt1) and HDAC activity (Clr3 and Sir2), respectively, to re-establish heterochromatin characteristics following replication[109–113]. Whereas experimental evidence substantiates this model in yeast, whether RNAi is involved in heterochromatin maintenance in mammals is unclear[114–116]. Although pericentric repeats are transcribed[117,118], not every component of the fission yeast RNAi machinery, such as RNA-dependent RNA polymerase, which serves in the post-transcriptional amplification of siRNA production, has been identified in mammals[119].

Mice. As in fission yeast, mouse pericentric heterochromatin is enriched in HP1 proteins, the binding of which is dependent on H3K9me3 as well as an unidentified structural RNA component[120,121]. Although H3S10 phosphorylation occurs on entry into mitosis in mammals[122,123], some HP1 is retained during mitosis and, in contrast to fission yeast, it is enriched in heterochromatin domains in G1 phase[124,125]. Cell cycle regulation of the transcription of pericentric repeats

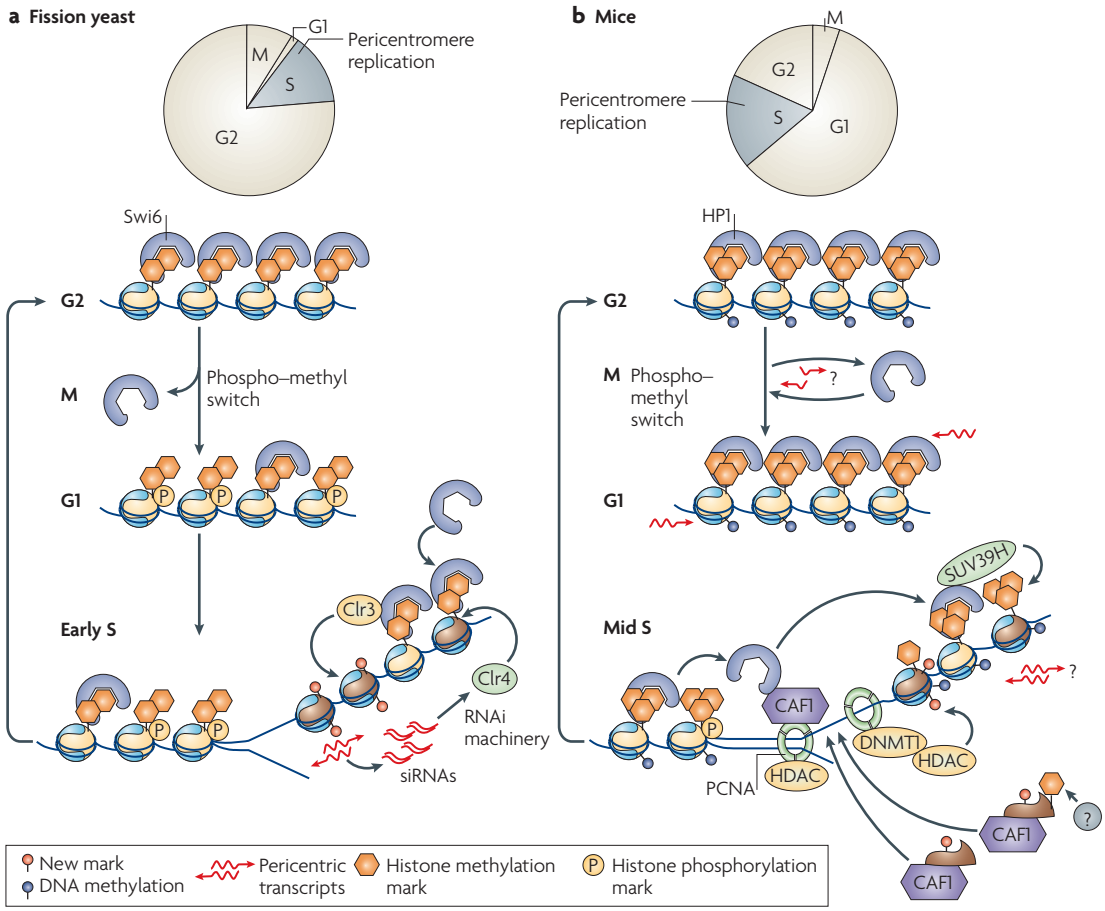


Figure 5 | **Maintaining pericentric heterochromatin in fission yeast and mouse.** Cell cycle profiles and the timing of pericentric heterochromatin replication differ between fission yeast and mammals. **a** | In G2 phase in fission yeast, pericentric hetrochromatin is enriched in dimethylated histone H3K9 (H3K9me2), which provides a binding site for the heterochromatin protein 1 (HP1) homologue Swi6 (REF. 69). On entry into mitosis, phosphorylation of H3S10 leads to reduced Swi6 association. This is termed the phospho–methyl switch[105–107]. Centromeric repeats are transcribed after centromere replication in early S phase and after dilution of histone marks[106,107]. Transcripts are processed by the RNA interference (RNAi) machinery into small interfering RNAs (siRNAs). The RNAi machinery recruits the histone methyltransferase Clr4 (the Suv39 homologue)[109]. Deacetylation by Clr3 and H3K9 dimethylation by Clr4 lead to the restoration of Swi6 binding and to silent heterochromatin maintenance[113]. **b** | Pericentric heterochromatin in mice contains methylated DNA and H3K9me3, which is bound by HP1 (REF. 70). The extent to which HP1 is disrupted by the phospho–methyl switch and how HP1 is restored in G1 phase is unclear[122–125]. Centromeric transcripts accumulate in mitosis and in G1–early S phase[126]; however, a direct role for RNA in heterochromatin maintenance in mice is lacking. Maintenance of pericentric heterochromatin occurs through the concerted action of DNA and histone modifiers and histone chaperones: DNA methylation is maintained by DNA methyltransferase 1 (DNMT1), which, together with proliferating cell nuclear antigen (PCNA), recruits histone deacetylase activity[78,81,82]. Chromatin assembly factor 1 (CAF1; also known as CHAF1) ensures histone H3.1 deposition and HP1 inheritance by the transfer of parental HP1 to daughter strands[128,130], where it is maintained by a self-perpetuating loop that involves SUV39H1 (also known as KMT1A)[67–70,181]. H3.1 can be monomethylated at residue K9 before deposition, serving as a substrate for further modification in chromatin[55]. HDAC, histone deacetylase.

was also documented in mice[126]. Two RNA species were identified: a short species that accumulates specifically in mitosis and another species of variable size that accumulates in G1 phase and peaks at G1–S phase. Whether the short pericentric transcripts have a role in HP1 dynamics during mitosis is unknown. However, the transcription of the longer species was found to cease before replication of heterochromatin domains, which renders a direct role for pericentric transcripts in post-replicative maturation of heterochromatin in mouse unlikely[126].

By contrast, the transmission and silencing of heterochromatin in mice could be ensured by mutual reinforcement between the inheritance of DNA and histone modifications at the replication fork (FIG. 5b). The maintenance of heterochromatin in mammals requires DNA methylation, histone deacetylation, H3K9 trimethylation and the transmission of HP1 proteins to the daughter strands. As discussed above, DNMT1 is enriched at pericentric heterochromatin in mid-S phase[127], and both PCNA and DNMT1 recruit HDAC activity. CAF1 is present in two mutually exclusive complexes, either

Small interfering RNA
A short, non-coding RNA (~22-nt long) that is processed from longer double-stranded RNA by the RNA interference machinery. Such non-coding RNAs confer target specificity to the silencing complexes in which they reside.

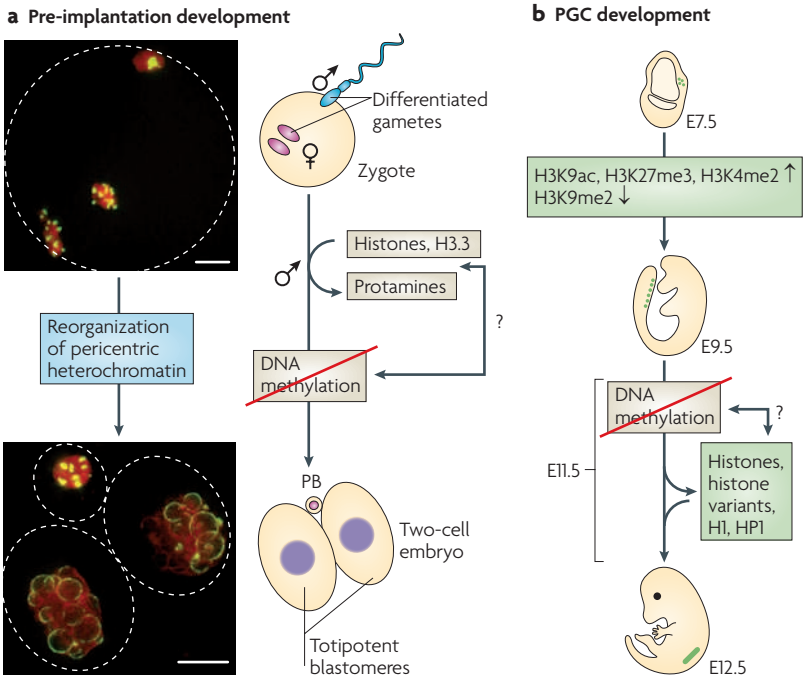


Figure 6 | **Reprogramming during development and the fate of epigenetic marks.** **a** | Pre-implantation development. At fertilization, two differentiated gametes unite in a single cytoplasm to form the zygote. Reprogramming during the first cell cycle establishes totipotent blastomeres at the two-cell stage. DNA methylation is present in both gametes. The maternal genome (pink) is organized into chromatin, whereas the paternal genome (blue) associates with protamines. Protamines are exchanged with hyperacetylated histones and H3.3 in the paternal genome[156,182,183], followed by DNA demethylation in a replication-independent manner[138,139]. Maternal and paternal pericentric heterochromatin undergo changes in higher-order organization and nuclear positioning during the reprogramming process, as shown here by DNA fluorescence *in situ* hybridization (FISH) for pericentric satellites (green)[136,141]. DNA is counterstained in red. Scale bar, 10 μm. **b** | Primordial germ cell (PGC) development. PGC reprogramming to pluripotency occurs in two waves. First, extensive changes in histone modifications occur (embryonic day 7.5 (E7.5)–E9.5). Second, during E11.5, DNA methylation imprints are erased[144,145], probably by a mechanism that implicates DNA repair[143]. This entails a significant reduction in histone marks, histone variants, histone H1 and heterochromatin protein 1α (HP1α) and HP1β (also known as CBX5 and CBX1, respectively), as well as substantial reorganization of pericentric heterochromatin[143]. General loss of DNA methylation persists but most histone modifications and heterochromatin organization are re-established by E12.5 (REF. 143). PB, polar body.

with histones or with HP1 (REFS 59,128), and is thought to ensure HP1 inheritance at the replication fork[128,129] by the transfer of the HP1 that is present before the fork to the newly formed chromatin[12,128,130]. Indeed, progression through mid S-phase is blocked by the mutation of the HP1-binding site in the p150 subunit of CAF1 (REF. 130), the knockout of which in mice is lethal[131]. Further retention of HP1 in heterochromatin involves both self-association of HP1 proteins and their interaction with SUV39H1 (also known as KMT1A), the major Lys methyltransferase that is responsible for trimethylation of H3K9 (REFS 67,68). The recruitment of SUV39H1 by HP1 is thought to create additional HP1-binding sites, and thereby forms a self-sustaining loop[69,70]. This mechanism could potentially also ensure HP1 binding to pericentric heterochromatin after destabilization in mitosis.

Phospho–methyl switch
The phosphorylation of histone H3S10 during late G2 phase and mitosis interferes with the binding of heterochromatin protein 1 to the adjacent methylated H3K9 residue.

Chromocentre
A cluster of constitutive heterochromatin from different chromosomes that is formed during interphase.

A fraction of CAF1 can also interact with MBD1 and SETDB1 during replication of heterochromatin[79,80]. Therefore, it can be envisaged that SETDB1 methylates H3 before deposition during the replication of DNA methylation- and MBD1-rich heterochromatin. Indeed, analysis of post-translational modifications of non-nucleosomal H3.1 reveals that monomethylation of H3K9 (H3K9me1) is the only methylation mark before deposition[55]. Once incorporated into chromatin, H3K9me1 could function as a substrate for further methylation by SUV39H1 (REF. 55), suggesting that chromatin domains, such as heterochromatin, can be pre-marked during histone deposition for further maturation at a later point.

Another important characteristic of mouse pericentric heterochromatin domains is their ability to cluster into higher-order structures called chromocentres (BOX 2). This higher-order organization is also challenged during DNA replication. Surprisingly, the domain does not undergo major rearrangements, as seen in 4′,6-diamidino-2-phenylindole (DAPI) staining (BOX 3), and HP1 is not visually displaced from heterochromatin during S phase[78]. Instead, pericentric heterochromatin replication takes place in specific duplication bodies, in which DNA is pulled to the periphery and is replicated. Following chromatin assembly, the DNA is re-internalized into the domain[128]. With chromatin disruption restricted to the periphery of the heterochromatin domain, large-scale structural changes are avoided and even association between heterochromatin domains from different chromosomes could be maintained during the replication process. Proper maturation of newly replicated chromatin following replication also affects the higher-order organization of pericentric heterochromatin domains. Exposure of mouse cells to the HDAC inhibitor TSA induces reversible hyperacetylation and chromocentre declustering[132]. Clustering of heterochromatic domains is also observed in fission yeast[133], in which the association of telomeres depends on a functional RNAi machinery and heterochromatin maintenance[111].

Pericentric heterochromatin is a typical example of constitutive heterochromatin and is defined — as opposed to facultative heterochromatin — to respond to the production of heterochromatin in the same way on both homologous chromosomes[134,135]. However, our recent understanding of how pericentric heterochromatin is established during development makes this distinction much less pronounced[136,137]. In the future, it will be important to evaluate whether the basic principles of the maintenance mechanisms described above apply to other heterochromatic regions.

Inheritance or reversibility?

Although the inheritance of epigenetic marks during the cell cycle provides a means of stably maintaining a cell lineage, epigenetic information is reversible in nature. The extensive reprogramming of epigenetic marks that is observed during early mammalian embryonic development and during primordial germ cell (PGC) development highlights the intrinsic reversibility of epigenetic states (FIG. 6).

During pre-implantation development, the highly specialized, differentiated gametes that are united in a single cytoplasm at the moment of fertilization face the challenge of forming a totipotent zygote, from which all cell types of an organism differentiate. The return to totipotency takes place in one cell cycle and involves differential DNA methylation and histone modification changes in paternal and maternal genomes[138–140], as well as large-scale chromatin reorganization, for example, of pericentric heterochromatin domains[136,141] (FIG. 6a). One should note, however, that sperm, although highly specialized, already seems to be largely reprogrammed when considering promoter methylation[142].

Major reprogramming also takes place in PGCs and involves the erasure of parental imprints, allowing the formation of germ cells and the setting of gamete-specific imprints. In a first wave of reprogramming, PGCs repress the somatic gene expression programme to acquire pluripotency characteristics[143]. In a second wave, PGCs erase DNA methylation imprints[144,145] and subsequently undergo significant changes in histone variants, modifications and chromatin-binding proteins, as well as the reorganization of pericentric heterochromatin[143,145] (FIG. 6b). Some chromatin changes are transient but the loss of DNA methylation imprints persists, and is a prerequisite for the proper development and the imposition of gamete-specific methylation marks.

A number of mechanisms have been proposed that contribute to the reprogramming of candidate epigenetic marks. Histone methylation marks that were long thought to be stable can be removed by passive dilution, histone exchange, controlled histone proteolysis or active demethylation that is catalysed by histone demethylases[146]. DNA methylation can be removed either by passive or active processes. DNA demethylation of the maternal genome during pre-implantation development is thought to be passive and can be explained by the dilution of DNA methylation during replication, by preventing DNMT1 from functioning at the replication fork[147,148]. The rapid DNA demethylation of the paternal genome following fertilization[138,139] and during PGC development suggests, however, that DNA methylation can be removed by active mechanisms[143,145,149,150]. Whereas in plants DNA glycosylases that are implicated in DNA demethylation have been characterized[151,152], the mechanisms and enzymatic activities that are responsible for DNA demethylation in mammals — although potentially linked to DNA repair — are controversial[42,43,149,153–155]. Another issue concerns the interrelationship between DNA demethylation and chromatin dynamics and whether chromatin changes are required to allow DNA demethylation to occur or whether DNA demethylation entails chromatin reorganization. An argument for the second possibility is the observation that, during PGC development, chromatin changes can be detected following DNA demethylation[143], whereas in the zygote, DNA demethylation follows protamine histone exchange in the paternal genome[139,156].

The possibility to experimentally reprogramme a differentiated nucleus, by exploiting the reprogramming potential of an oocyte, further highlights the reversibility of epigenetic states. Even a cancer cell has been successfully reprogrammed, thereby providing proof for the epigenetic nature of changes at the origin of cellular transformation in some cancers[157]. The successful reprogramming of somatic cells in culture to induced pluripotent stem (iPS) cells by the expression of a selection of transcription factors is a major breakthrough on the way to exploit the reversibility of epigenetic states for therapeutic use[158,159]. This system might allow the study of how changes in epigenetic states arise when induced by the expression of these reprogramming factors. However, any analysis might be complicated by the requirement for several rounds of replication and cell divisions and the stochastic manner in which the changes occur. Rather, the use of cell fusion experiments[160] might prove highly informative in understanding the molecular mechanisms that govern reprogramming and how they are manifested and perpetuated at the level of chromatin organization.

Concluding remarks

During the past few years, great progress has been made in identifying key candidates for epigenetic marks and the mechanisms that ensure their inheritance and reversibility. Molecular players have been characterized that, alone or in combination, function to impose and remove marks on histones and DNA, and insight has been gained into the metabolism of chromatin-associated nuclear RNA and the principles that control nuclear compartmentalization.

It is now clear that complex mechanisms operate at the replication fork to ensure the epigenetic inheritance of DNA methylation, DNA- and chromatin-binding factors, histone modifications and other factors that contribute to higher-order structures. The corresponding machinery likely integrates information of the local chromatin environment, and thereby leads to mutual reinforcement of inheritance of the different marks. Simple models have been built to integrate these parameters. To integrate the increasing complexity of nuclear organization, which we are beginning to unravel, these models have to be evaluated in different regions of the genome and at specific moments during development. In certain cases, faithful inheritance of all epigenetic marks at the fork might not necessarily be desired, and consequently replication presents a window of opportunity to induce a change in epigenetic state.

In addition, not all marks are directly imposed at the replication fork, as chromatin undergoes further dynamics that are uncoupled from the replication process. Certain histone variants, such as CENP-A, are inherited in a replication-independent manner, which brings other phases of the cell cycle into the spotlight. Furthermore, non-dividing quiescent cells with a long lifetime, such as neurons, stably maintain their cellular identity. How this is achieved in the face of cellular renewal remains to be investigated.

Although we have mostly discussed mitotic inheritance of epigenetic marks, a few examples in mammals

suggest that epigenetic states that are established during the life of an organism could be passed on to the next generation[161–164]. The candidate epigenetic marks that are transmitted through meiosis and are responsible for this transgenerational inheritance, and the influence of environmental factors including diet (for example, the level of methyl donors) on transmission, remain matters of debate[165–167].

There is also evidence for inheritance that does not rely on chromatin, but might instead involve RNA or cytoplasmic factors[3,168–171]. We should thus remain open to the identification of new heritable marks that can impact on gene expression and the developmental programme of an organism. Furthermore, it is likely that future progress will also reveal alternative mechanisms of inheritance that are uncoupled from the disruptive event.

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DATABASES

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CAF1 | CENP-A | DNMT1 | G9a | H3.1 | H3.3 | H4 | HDAC1 | HDAC2 | HP1 | MBD1 | NP95 | PCNA | Rtt106 | SETDB1 | Swi6

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