Heterochromatin—many flavours, common themes

Jeffrey M. Craig

Summary
Heterochromatin remains condensed throughout the cell cycle, is generally transcriptionally inert and is built and maintained by groups of factors with each group member sharing a similar function. In mammals, these groups include sequence-specific transcriptional repressors, functional RNA and proteins involved in DNA and histone methylation. Heterochromatin is cemented together via interactions within and between each protein group and is maintained by the cell's replication machinery. It can be constitutive (permanent) or facultative (developmentally regulated) and be any size, from a gene promotor to a whole genome. By studying the formation of facultative heterochromatin, we have gained information about how heterochromatin is assembled. We have discovered that there are many different architectural plans for the building of heterochromatin, leading to a seemingly never-ending variety of heterochromatic loci, with each built according to a general rule. ***BioEssays*** **27:17–28, 2005. © 2004 Wiley Periodicals, Inc.**

Introduction

This review will present a synthesis of what we know about the nature of heterochromatin today. It summarises the common constituents and properties of heterochromatin learned from many eukaryotes and exemplified in mammals. The most important themes of this review are that heterochromatic loci may appear different but are all based on similar groups of factors and that variety comes from variation within the groups of factors and in the order in which the factors are assembled. The factors involved in building heterochromatin will be introduced, from the foundations of sequence-specific transcriptional repressors and non-coding RNA to the building factors involved in DNA and histone methylation. The interacting trio of histone methyltransferase enzyme, methylated histone substrate and HP1 or Polycomb homologue will be introduced as the major endpoint of heterochromatin (Fig. 4). The role of proteins involved in DNA replication in the maintenance of replication will also be discussed.

Chromosome Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, Melbourne, Victoria 3052, Australia. E-mail: jeff.craig@mcri.edu.au
Funding agency: The National Health and Medical Research Council of Australia.
DOI 10.1002/bies.20145
Published online in Wiley InterScience (www.interscience.wiley.com).

Abbreviations: CAF-1, chromatin assembly factor 1; CENH3, centromere-specific histone H3 homologue; Chromodomain, chromosome organisation modifier domain; DNMT, DNA methyltransferase; EZH, Enhancer of zeste histone methyltransferase; homologue; H3-K9/K27, lysine at position 9/27 of histone H3; HDAC, histone deacetylase; HMT, histone methyltransferase; HP1, heterochromatin protein 1; KAP-1, Krüppel-associated box -associated protein-1 (also known as Tif1β); MBD, methyl-binding domain; MeCP, methyl CpG-binding protein; NuRD, nucleosome remodelling and histone deacetylation; ORC, origin recognition complex; PCNA, proliferating cell nuclear antigen; RNAi, RNA interference; SET domain, domain shared by the *Drosophila* proteins Su(var)3-9, Enhancer of zeste and Trithorax; siRNA, short interfering RNA; Xi, inactive X; Xist, X-inactive specific transcript.

Heterochromatin was discovered because it behaved differently to "true" (eu)chromatin by remaining visibly condensed throughout the cell cycle.[1] Later, it was discovered that heterochromatic chromosome regions contained repetitive DNA. Much of what we know about heterochromatin was learned from studies of the polytene chromosomes of *Drosophila*, which exhibit large bands of pericentric heterochromatin containing very few expressed genes. Located elsewhere, among the euchromatic arms of these polytene chromosomes, are found smaller bands of proteins belonging to the Polycomb group (PcG). These proteins are involved in silencing "master genes" involved in major developmental processes. Furthermore, in a phenomenon known as position effect variegation, genes can be silenced when juxtaposed next to pericentric heterochromatin via a spreading mechanism.[2] Though observed in *Drosophila*, there is evidence that most of these properties are exhibited one way or another in most eukaryotes. Many functionally important heterochromatin factors are conserved in *Drosophila*, fission yeast and budding yeast.[3,4] Vertebrate heterochromatin, on which this review will concentrate, is supplemented with CpG methylation and associated proteins.[5]

The many shapes and sizes of heterochromatin

Constitutive heterochromatin remains condensed throughout an organism's lifespan and facultative heterochromatin is assembled when needed to permanently silence genes. Examples of constitutive heterochromatin are the large bands of pericentric satellites present next to the centromeres of human and mouse chromosomes. Telomeres also exhibit many of the properties of constitutive heterochromatin, but contain a smaller amount of it. Facultative heterochromatin is

developmentally regulated and spends some of its life as euchromatin. An example is the mammalian inactive X (Xi), in which a random, female X chromosome is silenced at an early stage of embryogenesis.[6] Facultative heterochromatin can also encompass the promotors of genes silenced during development.[7,8] This type of heterochromatin has previously been referred to as ''silent chromatin'',[3] ''intercalary'' heterochromatin[9] or heterochromatin-*like*.[7] In addition, most neocentromeres (centromeres arising de novo from ectopic locations) switch from a euchromatic to a heterochromatic state.[10,11] In this review, all of the above are referred to as heterochromatin because they share common molecular components. The variation within the components and the order in which they are assembled influence the specific nature of each heterochromatic locus.[4,12]

Common components of heterochromatin

The major components of heterochromatin are summarised in Fig. 1. Many of the factors can be grouped according to function: DNA methylation, histone methylation and replication. In addition to these factors, we have the most-likely candidates for initiation (foundation) of the process of heterochromatin formation: transcriptional repressors and functional RNA. There are also accessory factors, some of which interact with many groups of proteins. All of these factors establish a cooperative and self-reinforcing organisation within heterochromatin as evidenced by accumulated data revealing a multitude of interactions and associations between them (Figs. 1–3). Each factor will be discussed in turn followed by a summary of their place in the ''chain of command'' of heterochromatin formation and maintenance.

Foundation factors

Transcriptional repressors

DNA sequence-specific transcription factors, whether repressors or activators, act as the founders of chromatin change. After binding to gene promoters, they initiate the accumulation of activation- or repression-specific factors (Figs. 1 and 2). All transcriptional repressors possess DNA-binding motifs and one of the most common is the zinc finger.[13] Zinc fingers bind to three base-pair sites on one strand of the DNA in a sequence-specific manner. Proteins may have many such domains in tandem, increasing their capacity to bind a wide range of DNA sequences. The most-widely-studied partner for repressors is the corepressor, whose function is to recruit other heterochromatin factors to gene promotors. However, transcriptional repressors have also been shown in some cases to bind directly to other heterochromatin components (Figs. 1–3 and see below).

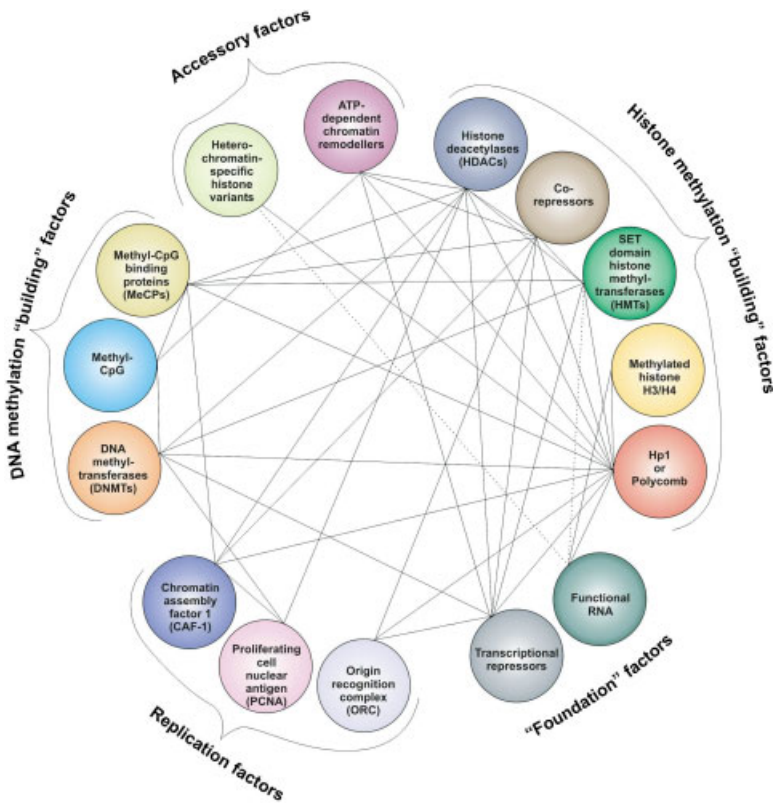


Figure 1. Interaction map of the major groups of proteins involved in the establishment and maintenance of heterochromatin. In addition to accessory factors and the ''foundation'' proteins of transcriptional repressors and functional RNA, proteins are classified through their association with DNA methylation, histone methylation and replication-coupled maintenance of heterochromatin. Interactions of any kind between any member of each group are indicated with solid lines and probable interactions with dotted lines. See text for references.

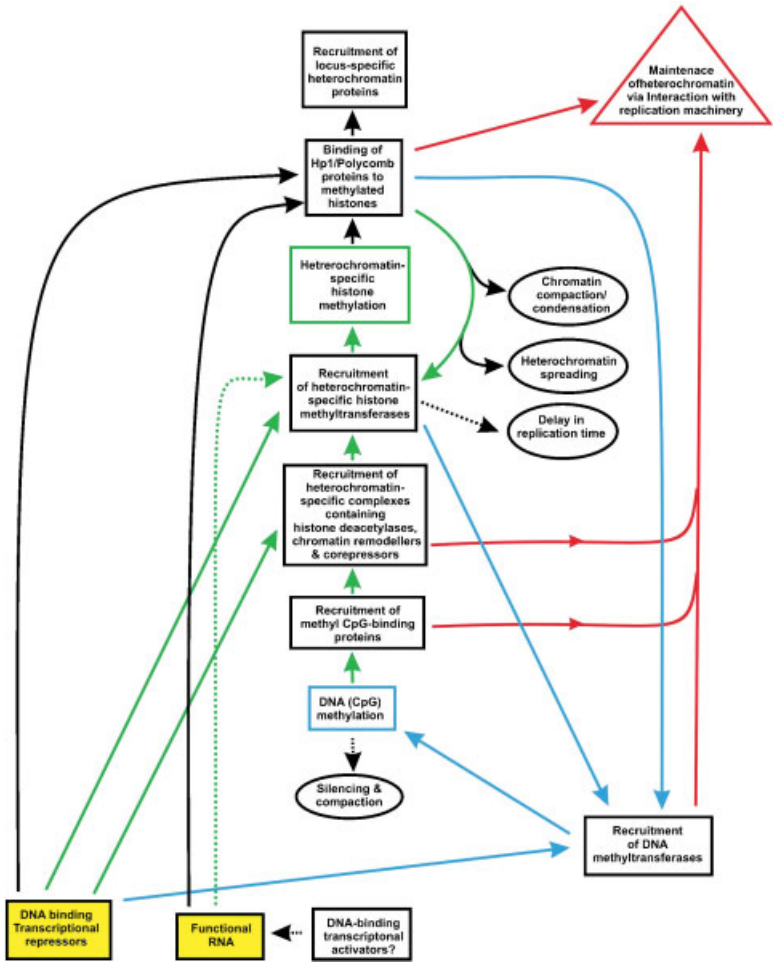


Figure 2. Summary of the pathways used to build and maintain mammalian heterochromatin, from the foundations of transcriptional repressors and functional RNA (shaded in yellow) through DNA methylation (blue arrows & box), histone methylation (green arrows and box), culminating in the binding of HP1 or Polycomb homologues and the recruitment of locus-specific heterochromatin proteins. Also shown are pathways involving heterochromatin factors that interact with the replication machinery (red arrows and triangle) and pathways to the physical properties of heterochromatin (circled). Transcriptional repressors can also directly recruit factors further along the pathway to histone methylation. Note also that functional RNA could be induced by sequence-specific transcriptional activators and that CpG methylation could lead directly to silencing and compaction (broken arrows). See text for references.

Noncoding, functional RNA

Noncoding, functional RNAs have been shown to be involved in genomic imprinting[8] and X inactivation.[6] However, a far-more-widespread phenomenon, RNA interference (RNAi) is emerging as an essential mechanism in the establishment of eukaryotic heterochromatin from yeast to mammals[14,15] (Figs. 1 and 2). RNAi at heterochromatin sites involves the expression of duplex-forming, overlapping sense and antisense RNA transcripts followed by on-site processing and amplification into short interfering RNAs (siRNAs), which induce histone methylation and subsequent heterochromatin formation.[16]

Henikoff and colleagues[16] have proposed that RNAi-initiated heterochromatin assembly has evolved to silence ectopically expressed or parasitic sequences within heterochromatin which if unchecked could disrupt heterochromatin structure and function. Perhaps RNAi has evolved to silence all foreign transcripts in eukaryotes, especially those repeated tandemly. This would explain why RNAi is seen in fission yeast and not budding yeast, which has a far lower repeat content and lacks heterochromatic, centromeric repeats.

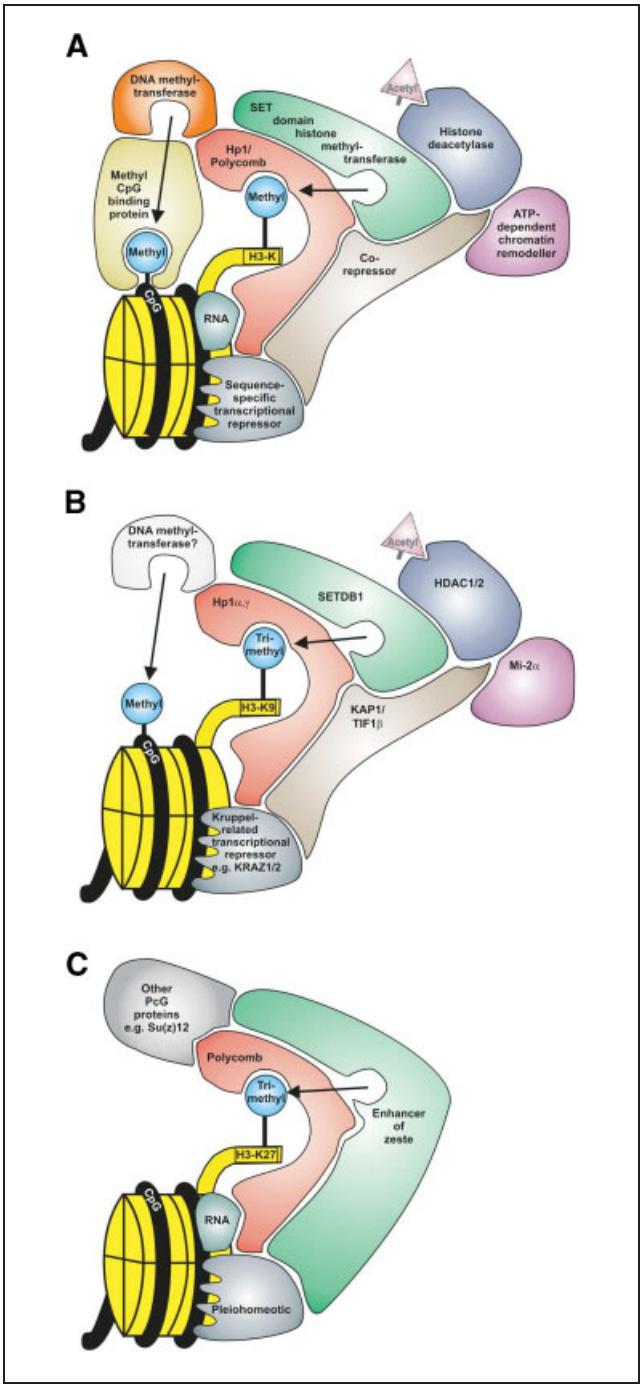
It is also worth noting that, in plants, RNAi can directly induce DNA methylation,[17] but no such direct link has been established in mammals. It is also worth considering that to transcribe an RNA, a transcriptional activator is usually needed. No candidates for such an activator are apparent but there is a precedent for fission yeast centromeres. The fission yeast zinc finger protein Ams2 binds to the same region of DNA as the centromere-specific histone CENH3 and is required for its binding to DNA.[18]

Building factors linked to histone methylation

Corepressors

The most-widely-studied binding partner for transcriptional repressors is the corepressor, whose function is to recruit one or more heterochromatin factors to gene promotors. Corepressors have also been shown to be present at

pericentric heterochromatin and can play a role in chromosome segregation.[19,20] The relationship between repressor and corepressor has been well studied for the multitude of zinc finger proteins containing a Krüppel-associated box (KRAB) domain.[21] KRAB domain proteins can recruit the corepressor KAP-1, which in turn recruits other heterochromatin proteins (Fig. 3B). Corepressors can also be a component of or associate with macromolecular complexes with histone deacetylase activity[22] (see below). Some corepressors are more well known for their other functions, for example, the tumour suppressor retinoblastoma (Rb) recruits many components of heterochromatin to the E2F family of repressors.[23]



Histone deacetylases and their complexes

Histone deacetylases (HDACs) lie at the heart of heterochromatin pathways and can be recruited by transcriptional repressors/corepressors and by proteins that bind to methylated DNA (Figs. 1–3 and see below). Levels of histone acetylation are in constant flux; histones newly deposited after DNA replication are acetylated and modifications may be added or subtracted at any time during the cell cycle.[24] HDACs, by mechanisms outlined below, can be targeted to newly deposited histones ensuring prompt deacetylation at appropriate residues. HDACs can form homodimers and heterodimers and can interact with many other heterochromatin proteins[25] (Fig. 1).

HDACs also form macromolecular complexes with other heterochromatin components in a similar way to and often in combination with, ATP-dependent chromatin remodellers (see below). The two major HDAC complexes are SIN3 (named after the SIN3 corepressor which is a central component) and NuRD.[22,25] Both complexes share HDAC1 and HDAC2. Importantly, subunits are evolutionally conserved,

Figure 3. Diagrammatic representation of selected pathways to heterochromatin. **A:** The general interactions involved in setting up heterochromatin via histone and DNA methylation using information reviewed in the text and illustrated in Figs. 1 and 2. **B,C:** Specific examples of interactions between known proteins at specific sites of heterochromatin formation. For clarity, not every interaction is represented. **A:** A transcriptional repressor binds to a specific DNA sequence and recruits a corepressor and/or HP1/Polycomb homologues, which can also be recruited by RNA. The corepressor recruits an chromatin remodeller that loosens DNA–histone associations within the nucleosome, HDACs that remove the acetyl group from a lysine residue on the tail of histone H3/H4 and SET domain HMTs that add a methyl group onto the same tail. The HMT and methylated lysine residue recruit HP1 or Polycomb homologues. Finally, HP1 recruits DNA methyltransferases, which methylate CpG residues within DNA and thereby attract methyl CpG-binding proteins. Not shown here is the reverse pathway from DNA methylation to histone methylation. **B:** The known factors recruited to gene promotors by the mammalian Krüppel-related transcriptional repressors resulting in the trimethylation of histone H3-K9[98] and **C:** The factors known to be recruited by the *Drosophila* PcG transcriptional repressor pleiohomeotic.[108] In the latter case, the SET domain HMT Enhancer of zeste (EZ) can bind directly to the DNA-binding protein Pleiohomeotic. Both EZ and Polycomb (which also binds RNA) recruit other PcG proteins to the DNA. Note that there is no DNA methylation in *Drosophila*.

underlining their functional importance in heterochromatin. HDAC complexes can be targeted by transcriptional repressors, corepressors and proteins that bind methylated DNA.[25] Combinations of HDAC complex-associated factors enable different loci to have complexes tailored to meet their specific requirements.[22]

Histone methyltransferases

Histone methylation at lysine residues is central to epigenetic regulation of gene expression and is carried out by histone methyltransferases (HMTs).[21,26,27] Contrary to the situation with histone acetylation, histone methylation can be associated with transcriptional activation or repression. Most HMTs (and so far all HMTs associated with repression) contain a SET domain (named after three *Drosophila* HMTs: Su(var)3-9, Enhancer of zeste and Trithorax). Histone residues that have been shown involved with repression include histone H3 lysine 9 (H3-K9), H3-K27 and H4-K20.[21] Furthermore, different HMT enzymes can modify the same lysine and lysines can accommodate one to three methyl residues.[21] The variety afforded by these combinations is thought to be central to creating a "histone code" to mark out different genomic loci for different combinations of factors necessary for the appropriate function of chromatin regions[26] (Fig. 4).

The most-widely-studied histone modification is methylation of H3-K9, catalysed by the HMT Suv39h. Suv39h has two mammalian orthologues, Suv39h1 and Suv39h2, and these take on much of the responsibility for methylation of H3-K9 at pericentric heterochromatin[28] (Fig. 4B). Suv39h1 is also associated with genomic imprinting.[29] At the promoters of many silent genes, H3-K9 methylation can be performed by Suv39h or other SET domain HMTs[21] (Figs. 3A,B, 4C).

Trimethylation of H3-K27 is catalysed by the mammalian HMT Enhancer of zeste homologue (EZH2; known as Enx1 in the mouse), which also exhibits an ability to trimethylate H3-K9[27] (Fig. 4D,E). EZH2-catalysed trimethylation of H3-K27 and probably dimethylation of H3-K9 is associated with setting up the silencing of the mammalian Xi[30] (Fig. 4J). However, after setup of the Xi, EZH2 is lost but methylated H3-K9 and H3-K27 remain (Fig. 4K). The maintenance HMT for H3-K9 is probably G9a but the maintenance HMT for H3-K27 is currently unknown (Fig. 4G).[27] EZH2-catalysed trimethyl H3-K27 is also associated with genes silenced by the Polycomb group (PcG) of proteins (Fig. 4E).[31] In addition, H4-K20 can be monomethylated or trimethylated by the HMTs PR-Set7[21,32] and Suv4-20h1/2[33] respectively (Fig. 4F,G).

Intriguingly, methylation of some lysine residues is dependent on the covalent modification of other residues.[21,26,32] However, the mechanisms behind this so-called "epigenetic crosstalk" have yet to be fully determined. Histone methylation could also be more of a lasting covalent modification than histone deacetylation. Whereas histone acetyltransferases and histone deacetylases exist, no histone demethylase has yet been identified. It is likely that either methylated histones are diluted after loss of HMT activity or, more likely, that they are replaced by variant histones (see below).

HP1 and Polycomb

HP1 and Polycomb are adapter molecules that bring together different heterochromatin proteins in macromolecular complexes. They are structural components of heterochromatin, bind methylated histones and the HMTs responsible for their methylation and share a region of homology—the chromodomain.

HP1 is highly conserved in structure and function from fission yeast to mammals. It is associated with many forms of heterochromatin including centromeres, telomeres, silent gene promoters and triplet repeat expansions, with the mammalian Xi being a notable exception.[34–36] The interaction central to heterochromatin formation and maintenance is the three-way interaction between HP1 or Polycomb, methylated lysine residues and HMTs[37] (Fig. 4A). The most-widely-studied trio of this kind is the one present at mammalian pericentric heterochromatin: HP1, Suv39h and H3-K9 (Fig. 4B). However, the HMT–methylated lysine–HP1/Polycomb trio is likely to be at the heart of all heterochromatic loci (Fig. 4C–K).

HP1 binding to chromatin is also dependent on an RNA component and HDACs,[38] most likely due to the necessary removal of an acetyl group from H3-K9 prior to methylation. HP1 self-dimerises and this enables it to recruit HDACs and Suv39h to adjacent nucleosomes to deacetylate and methylate H3-K9, respectively. Together with the crosslinking effect of internucleosome HP1 dimerisation, this can lead to the formation of a higher order structure and/or a spreading of heterochromatin along a chromatin fibre (see below). HP1 can also attract a whole host of other proteins to heterochromatin including proteins necessary for sister chromatid cohesion[34,39,40] (Fig. 4).

Mammals have three isoforms of HP1—HP1α, HP1β and HP1γ. Isoforms can exhibit localisation within cytologically identifiable heterochromatin and/or euchromatin and exhibit differences in nuclear localisation throughout the cell cycle[41] (Fig. 4B,E,I). The association of HP1 proteins with chromatin is a dynamic process, HP1 being rapidly exchanged.[42] These findings could imply that, although HP1 helps maintain a refractive heterochromatic structure, it could enable chromatin to rapidly change its transcriptional status.

Polycomb group (PcG) repressor proteins, first described in *Drosophila*, form multimeric complexes that are required for developmentally regulated silencing of genes determining cellular fate.[7] They are a heterogeneous group of proteins and most members are conserved in metazoa. The founding

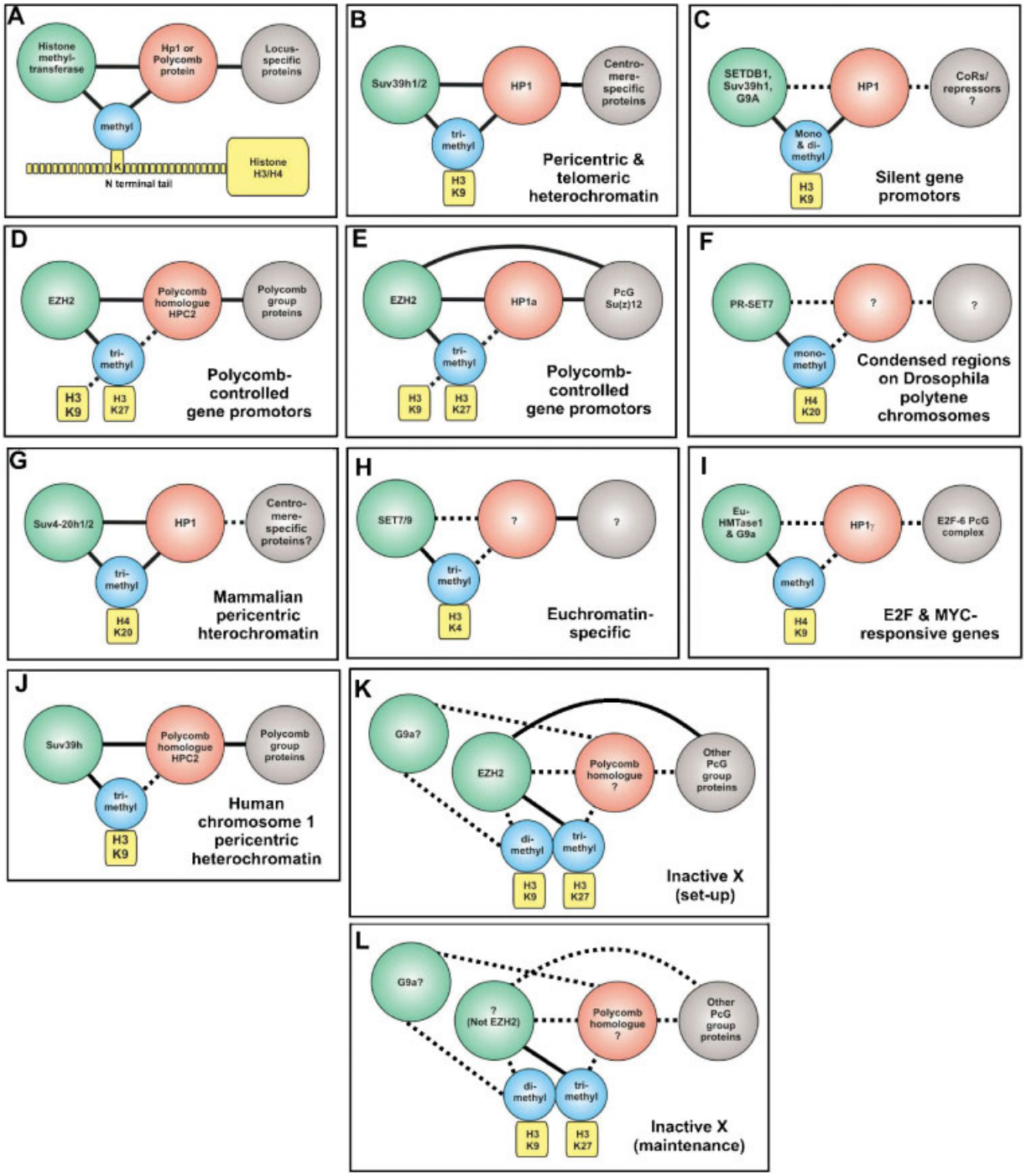


Figure 4. The histone code for mammalian heterochromatin as written by the trio of mammalian SET domain HMT–methylated histone H3/H4–HP1/Polycomb. Yellow boxes represent histone lysine residues, blue circles represent methyl groups, green circles represent histone methyltransferases and red circles represent HP1 or Polycomb homologues. Question marks indicate unknown information. Protein–protein interactions are indicated by solid lines; probable interactions based on other interactions and/or from *Drosophila* by dotted lines. Generic interactions are shown in **A** and known locus specific interactions in **B–L**. For references see text.

member, Polycomb, has a chromodomain. In *Drosophila* and mammals, PcG proteins work together in promoter-binding complexes, which may contain a SET domain HMT and/or a histone-binding Polycomb homologue.[7,8] These proteins in turn bind to corepressors, HDACs, chromatin-remodelling proteins and RNA.[7,43,44] For example, the human PcG HMT EZH2 methylates H3-K27 and binds Polycomb homologue HPC2 (Fig. 4D). Interestingly, HPC2 can also bind to Suv39h[45] (Fig. 4J), HP1 can attract PcG proteins to gene promotors[39,46] (Fig. 4E), and Polycomb can self-dimerise, further demonstrating the similarity between HP1- and Polycomb-associated complexes. This knowledge enables us to predict that a SET domain HMT and chromodomain protein will fill the gaps in our knowledge about the heterochromatin proteins that bind to the mammalian Xi, which does not bind HP1 isoforms (Fig. 4K,L).

Building factors linked to DNA methylation

Vertebrate DNA can be methylated at the cytosine of the dinucleotide CpG.[5,47,48] CpG residues that usually escape methylation are clustered in ''CpG islands'' at or near gene promotors. De novo methylation of CpG islands can occur during the formation of facultative heterochromatin, for example on the Xi, some imprinting centres, some permanently silenced genes, and genes methylated during ageing and carcinogenesis.[5,48]

Methylation is also responsible for silencing transposable elements and reducing transposon hyperactivity.[5] Methylation and subsequent heterochromatin formation within tandemly repetitive DNA are also thought to inhibit recombination between homologous repeats, which could otherwise lead to genome instability.[14]

DNA methyltransferases (DNMTs)

CpG dinucleotides are methylated by DNMTs. Mammals have two types of DNMT—the essential, de novo methyltransferases DNMT3A/B and a maintenance methyltransferase, DNMT1. DNMT3A/B are expressed at highest levels during the wave of de novo methylation in early embryogenesis.[5,48] DNMT3B is responsible for de novo methylation and the structural integrity of pericentric DNA, and for methylation of imprinting centres and CpG islands on the Xi.[8,49]

Methyl CpG-binding proteins (MeCPs)

Methyl CpG-binding proteins (MeCPs) form a major subset of a group of proteins that contain methyl CpG-binding domains (MBD). They play a major role in transcriptional repression by recruiting heterochromatin-specific proteins to methylated DNA. It has also been found that MeCPs prevent and even compete with the binding of *trans*-activating factors to methylated regulatory sequences.[47,50] MeCPs are found at pericentric heterochromatin, imprinting centres and the CpG islands on the Xi.[48] The major human MeCPs are MeCP2 and MBD1/2. These MeCPs also have a transcriptional repression domain that is needed to recruit heterochromatin-specific proteins such as the corepressors, HDACs and ATP-dependent chromatin remodellers.[47]

The interplay between DNA and histone methylation

The synergistic interdependence between DNA methylation and histone methylation may reinforce a heritable silent state.[51] As mentioned below, DNA methylation is more often than not an event downstream from other heterochromatin-specific changes. So what attracts the de novo DNMTs to silenced regions of the genome at this time? It has been proposed that a new heterochromatin state may ''inform'' the de novo methyltransferases to methylate a silenced promoter.[52] Recent evidence supports this hypothesis and suggests that the ''informer'' is HP1[53] (Fig. 3A). The reverse pathway has also recently been observed. MeCP2 was shown to tether histone methylation activity to a silent gene promoter[54] and a methylated transgene.[55] Further evidence of links between DNA and histone methylation come from findings that some HMTs also have methyl CpG-binding domains.[23]

Does DNA methylation ever come first?

The place of CpG methylation in the formation of heterochromatin has long been debated. It is generally accepted that, during normal development, methylation is secondary to other mechanisms of transcriptional silencing, and that DNA methylation may act as a stabiliser for inactive chromatin, having evolved as yet another heritable epigenetic mark in more complex genomes.[5,47] For example, methylation of the CpG islands on the Xi occurs after all other epigenetic events.[6] However, there are some cases where it looks like methylation does come first. Firstly, there are a small number of cases that demonstrate that methylation of a DNA sequence can directly interfere with interactions of that sequence with *trans*-acting factors and initiate silencing directly.[56,57] In addition, MeCP2 was shown to tether histone methylation activity to a methylated transgene[55] and methylation of imprinting centres can trigger heterochromatin formation.[8,48] Our knowledge of the mechanisms behind this is sketchy, but we do know that some proteins can bind specifically to unmethylated imprinting centres to prevent subsequent CpG methylation.[56]

In cancer, de novo CpG methylation accompanies gene silencing and precedes histone methylation.[58] But how do DNMTs methylate specific CpG islands if they are not sequence-specific? There are two main possibilities. The first assumes that methylation and demethylation of CpG islands are involved in a constant dynamic equilibrium whose balance may be tipped by gene activity.[59] The second is that DNMTs are targeted to promotors via transcriptional repressors. Cancer-specific overexpression of a repressor or under-

expression of an activator could lead to a repressor binding to a gene promotor and recruiting a DNMT. As DNMTs are overexpressed in a wide variety of cancers,[59] this may ensure a ready supply of these proteins at repressor-bound promotors. In non-cancerous cells, DNMT3A can be recruited to a promotor by a sequence-specific transcriptional repressor.[60] This suggests that this mechanism may be more widespread than is currently thought.

Accessory factors

ATP-dependent chromatin remodellers and their complexes

ATP-dependent chromatin remodellers can be recruited to chromatin by transcriptional repressors or corepressors (Figs. 1–3). They use the energy generated by hydrolysis of ATP to modify nucleosomes in a non-covalent manner to increase chromatin accessibility.[22,55] This loosens histone–DNA interactions, facilitating access to DNA by other factors including those involved in heterochromatin formation and DNA replication.[25]

Families of ATP-dependent chromatin remodellers are conserved throughout eukaryotes. They can exist in macromolecular complexes in combination with other remodellers or with a wide selection of other chromatin proteins including HDACs.[25,61] Mammalian chromatin remodellers have been shown to be essential for DNA methylation[62] and can recruit heterochromatin proteins to newly methylated DNA via an association with methyl CpG-binding proteins.[63]

Histone variants and linkers

Histone variants have homologies to canonical core histones but are generally not as ubiquitous. They represent a further mechanism for nucleosomes to differ from one another without covalent modification of core subunits.

Histone H2A variants MacroH2A1/2 have intrinsic transcriptional repression activity and are present on the Xi chromosome between DNA replication and cell division.[64] Macro H2A also interacts with HP1[35] and may bind RNA.[65] The histone variant H2A.Z is involved in the establishment of budding yeast silent mating type loci, where it is also responsible for restricting the spread of silent chromatin.[66] It is also involved in chromosome segregation in fission yeast and is present at pericentric heterochromatin after differentiation of the inner cell mass of mouse embryos.[67] H2A.Z colocalises with HP1α in differentiated embryonic cells where it is involved in generating a more compact chromatin structure and regularly spaced nucleosomes.[6,72]

Ahmad and Henikoff[68] showed that histone variant H3.3 can replace H3 and even meH3K9 by a replication-independent pathway. They also suggested that H3.3 could be incorporated in preference to H3 during early S phase, temporally separating active (early) and inactive (late) fractions of the genome. Replacement of methylated histones with histone H3.3 is currently the most-likely mechanism of disposing of methylation marks within a nucleosome.

The linker histone H1 and its variant histone H5 play a central role in higher-order folding of 30 nm fibres by clamping DNA fibres to nucleosomes and enabling the formation of higher-order structures.[69] In cell types with extensively repressed and compact genomes such as erythrocyte nuclei, H1 is replaced by H5 and the total amount of linker histones is elevated.

Are all heterochromatin proteins heterochromatin specific?

The simple answer is no. Some transcription factors can recruit corepressors or coactivators and some corepressors can also act as coactivators.[70,71] Even HP1 can function both in repression and activation[15] and is responsible for the expression of genes located within *Drosophila* pericentric heterochromatin.[72] DNA methylation is also not exclusively associated with heterochromatin. The methylation of some imprinting centres is associated with activity of the associated imprinted gene, which can be explained by a mechanism whereby methylation displaces *trans*-acting factors associated with repression.[56]

Pathways to heterochromatin

Though similar factors are involved in heterochromatin formation at different loci, there is no single pathway to heterochromatin formation (Figs. 2 and 3). A DNA-bound transcriptional repressor can recruit complexes containing corepressors, ATP-dependent chromatin remodellers and HDACs. Subsequent to HDAC-catalysed deacetylation of core histone tails, SET domain HMTs, recruited by many of the above factors and possibly functional RNA, methylate the same core histone tails at lysine residues. HP1 or Polycomb homologues are then recruited by HMT and methylated histone substrate (Fig. 3) with the help of functional RNA and, in some cases, transcriptional repressors. HP1 and Polycomb can recruit accessory proteins such as cohesins or other PcG proteins to perform locus-specific roles. DNA methylation is initiated by DNMTs and attracts MeCPs and this sequence of events can fit into heterochromatin formation in two ways. DNMTs can be recruited by transcriptional repressors (DNA methylation first) or by HMTs/HP1 (histone methylation first). All these factors are kept on the chromatin with the help of an epigenetic memory-transferring chromatin state from one cell cycle to the next and discussed below.

Heterochromatin maintenance and DNA replication

Once every cell cycle, genomes need to be faithfully replicated. During S phase, complementary strands of DNA are synthesised and histones are distributed between the

daughter strands. How does the chromatin state get passed on to daughter strands considering that newly deposited histones possess different modifications to the incumbent ones, and how are the "gaps" in the chromatin on the new strands filled? How are non-histone proteins duplicated? The answer lies in a general temporal difference between euchromatin and heterochromatin and in specific interactions between components of the replication machinery and chromatin complexes.

Late replication

In general, heterochromatin replicates later than euchromatin[40,73] with some exceptions.[40,74] Chromatin structure directly influences replication timing.[40,73] For example, patients with mutations in the *Dnmt3b* gene have hypomethylated CpG islands on the Xi chromosome accompanied by an earlier replication time, despite the presence of XIST RNA.[75] In addition, neocentromere formation, also associated with the acquisition of heterochromatin, is also associated with the appearance of domains of later-replicating DNA.[76] HDAC activity contributes to maintenance of a late replication time at imprinted loci,[77] at neocentromeres[78] and at other locations in mammalian cells.[73] It has been suggested that partitioning the genome into domains with specific replication times has evolved to help maintain order in increasingly complex metazoan genomes.[73] It is therefore not surprising that many chromatin proteins including DNMTs, HP1, HMTs and chromatin remodellers colocalise with late replicating DNA.[79] However, some proteins whose main function lies in DNA replication can also participate in replication-coupled heterochromatin assembly.

CAF-1, PCNA and the replication of heterochromatin

Central to the process of replication-coupled heterochromatin assembly are the interacting proteins chromatin assembly factor-1 (CAF-1) and proliferating cell nuclear antigen (PCNA).[80] CAF-1 is a complex of three subunits and responsible for the assembly of nucleosomes onto newly replicated DNA. PCNA is a sliding clamp that serves as a loading platform for many proteins involved in DNA replication and repair, and is deposited on DNA after passage of the replication fork. CAF-1 recruits acetylated histones H3 and H4,[81] MBD1[82] and HDAC complexes to DNA.[81] PCNA recruits the maintenance DNA methyltransferase DNMT1.[83]

The origin recognition complex (ORC) and the replication of heterochromatin

ORC is a six-subunit, DNA-binding complex necessary for initiation and temporal control of eukaryotic replication. ORC may also recruit proteins involved in the maintenance of heterochromatin.[84] ORC2 interacts with HP1 in *Drosophila* and *Xenopus*,[85] *Drosophila* ORC2 mutants show delocalisation of HP1 from pericentric heterochromatin,[86] and mammalian ORC1 can bind to transcriptional repressors.[87] Perhaps the ORC complex, by binding heterochromatin complexes, may help translate chromatin state into replication time.

Physical properties of heterochromatin

Heterochromatin has a number of physical properties. It can spread, it is has a highly condensed higher order structure, it tends to cluster to form nuclear compartments and it is often cytologically visible. The final section of this review will discuss the mechanisms behind these properties and make connections with the heterochromatin-specific factors mentioned earlier.

Spreading

As discussed above, the binding of HP1 to both Suv39h and methylated H3-K9 implies that HP1 can recruit Suv39h to methylate an adjacent nucleosome, thereby spreading heterochromatin. Heterochromatin spreading would therefore be limited by the nuclear pool of HP1, which is indeed the case with position effect variegation in *Drosophila*.[2] Lateral spreading of heterochromatin from a nucleating sequence has also been demonstrated in fission yeast.[88] For the small pockets of heterochromatin within euchromatin, the story may be slightly different. Domains of heterochromatin proteins present at silent gene promoters within euchromatin spread from the promotor, with the spreading limited by insulator and boundary elements.[89,90] Such spreading has also been found to be necessary for irreversible gene activation.[90]

Condensation

Heterochromatin is more condensed and self-associates more than bulk chromatin.[69] This is aided by regular spacing of nucleosomes and by histone variants, MeCP2[91] and the crosslinked, higher-order structures afforded by the dimerisation of HP1. The recent finding that HP1 may bind to two H3-K9 residues[92] underlines the latter's involvement in formation of higher order structures within heterochromatin. There is even evidence that HP1 dimers can form *in trans*, linking two separate chromosome loci,[93] which goes some way to explaining the mechanisms behind the clustering of heterochromatin domains in interphase. The tight packaging of chromatin is also evidenced by the finding that a "branched chain" antibody raised against four identical, linked, H3 peptides each dimethylated at H3-K9 is specific for pericentric heterochromatin, whereas an antibody raised against a linear peptide stains chromosomes uniformly.[28] Condensation of heterochromatin can also be aided by proteins such as MENT, which binds specifically to methylated H3-K9[94] and condensins, which can interact with DNMT3B.[95]

Compartmentalisation

The eukaryotic nucleus has dynamic but ordered substructures, with many protein components restricted to nuclear

compartments.[96] Clusters of interphase heterochromatin contain centromeres, pericentric heterochromatin and telomeres and are frequently found at the nuclear periphery.[97] The peripheral localisation of these clusters may be due at least in part to the interaction of HP1 with nuclear lamins.[35] Some but not all genes utilise these clusters of inactive chromatin as an additional layer of transcriptional control.[96,98–100] Silenced imprinted alleles are also located towards the exterior of a nucleus.[101] Interestingly, PcG proteins are found on the surface of condensed chromatin domains,[102] suggesting that they need only a limited access to heterochromatin factors, most likely because they recruit many PcG-specific factors and only rarely HP1. It is also important to note that not all components of silent heterochromatin compartments should not be considered as static, as HP1 has a dynamic association with heterochromatin compartments.[42]

Cytological visibility

Only the larger regions of heterochromatin manifest as cytologically observable metaphase chromosome bands. Mammalian pericentric heterochromatin can be seen on most human chromosomes as C- (centromere) bands but cannot be seen at neocentromeres, despite the presence of heterochromatin proteins.[78] High copy, non-centromeric tandemly repetitive heterochromatin was shown to manifest as C-band heterochromatin only when it exceeded 10.5–17.5 Mb in size.[103] Two neocentromeres, with 2.5–3 Mb of condensed chromatin and with as little as 100 kb of HP1[104] clearly fall short of this threshold.

An incredible variety of heterochromatin

Heterochromatin is not a uniform structure; it comes in all shapes and sizes from gene promotors, through bands of pericentric heterochromatin, the inactive X chromosome up to the whole genome of terminally differentiated erythrocyte nuclei. The similarities between these types of heterochromatin and the differences in size between heterochromatin domains have already been discussed, but how does heterochromatin vary in other ways?

This comes from the sequence-specific nature of functional RNA and transcriptional repressors,[105] from diversity within groups of factors such as HMTs (Fig. 4) and from the diversity in binding partners of each heterochromatin component. This diversity in used to generate heterochromatin differing in composition between different loci, tissues and stages of development. For example, the HP1γ isoform and Polycomb homologues are not found in pericentric heterochromatin[7,34,106] and most heterochromatin factors have members that are expressed only during embryogenesis. It is also important to reiterate that not all regions of heterochromatin contain the same set of methylated histones and it has been proposed that combinations of methylated lysines act as an index or code for separate heterochromatic loci.[26,107]

Conclusions

Heterochromatin is a biophysical entity built up from the foundations of sequence-specific transcriptional repressors and/or functional RNA. The two main pathways to building heterochromatin involve DNA methylation and histone methylation and participants in both of these processes interact with the replication machinery to enable the stable epigenetic inheritance of a heterochromatic state. Histone methylation can lead to DNA methylation and vice versa but there is much still to be learned about the situations in which these different pathways occur. The components of heterochromatin establish a cooperative and self-reinforcing organisation with some built-in redundancy and are maintained in a dynamic equilibrium. There is no strict order in the assembly of heterochromatin but there are general rules about the types of interactions involved, one of the most universal being the interacting trio of histone methyltransferase, methylated histone and HP1 or Polycomb proteins. This trio in turn can recruit locus-specific heterochromatin factors. These general rules apply to all heterochromatic loci. This information will help us fill in the missing pieces in the jigsaw of protein interactions at partially characterised heterochromatic loci. It will also help us predict the order in which heterochromatin is put together at these loci and to identify the transcriptional repressors that initiate heterochromatin formation and possibly the transcriptional activators that initiate the transcription of sequence-specific functional RNA. Truly, heterochromatin has many flavours but common themes.

Acknowledgments

Thanks to David Tremethick, Jacinta Ryan, Richard Saffery, Paul Kalitsis, Nick Wong, Huseyin Sumer and Andy Choo for their helpful comments. Apologies for all primary references not cited due to space constraints. The author is an associate of the Department of Paediatrics, University of Melbourne.

References

1. Heitz E. 1928. Das Heterochromatin der Moose. I Jahrb wiss Bot 69:726–818.
2. Wakimoto BT. 1998. Beyond the nucleosome: epigenetic aspects of position-effect variegation in *Drosophila*. Cell 93:321–324.
3. Richards EJ, Elgin SC. 2002. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. Cell 108:489–500.
4. Dillon N, Festenstein R. 2002. Unravelling heterochromatin: competition between positive and negative factors regulates accessibility. Trends Genet 18:252–258.
5. Bird A. 2002. DNA methylation patterns and epigenetic memory. Genes Dev 16:6–21.
6. Brockdorff N. 2002. X-chromosome inactivation: closing in on proteins that bind Xist RNA. Trends Genet 18:352–358.
7. Orlando V. 2003. Polycomb, epigenomes, and control of cell identity. Cell 112:599–606.
8. Rand E, Cedar H. 2003. Regulation of imprinting: A multi-tiered process. J Cell Biochem 88:400–407.
9. Zhimulev IF, Belyaeva ES, Makunin IV, Pirrotta V, Semeshin VF, et al. 2003. Intercalary heterochromatin in Drosophila melanogaster polytene chromosomes and the problem of genetic silencing. Genetica 117:259–270.

10. Amor DJ, Choo KH. 2002. Neocentromeres: role in human disease, evolution, and centromere study. Am J Hum Genet 71:695–714.
11. Craig JM, Wong LH, Lo AW, Earle E, Choo KH. 2003. Centromeric chromatin pliability and memory at a human neocentromere. EMBO J 22:2495–2504.
12. Martin DI. 2002. Activators antagonize heterochromatic silencing: reply to Eissenberg. Bioessays 24:102; author reply 103.
13. Urnov FD. 2002. A feel for the template: zinc finger protein transcription factors and chromatin. Biochem Cell Biol 80:321–333.
14. Grewal SI, Moazed D. 2003. Heterochromatin and epigenetic control of gene expression. Science 301:798–802.
15. Kellum R. 2003. Is HP1 an RNA detector that functions both in repression and activation? J Cell Biol 161:671–672.
16. Vermaak D, Ahmad K, Henikoff S. 2003. Maintenance of chromatin states: an open-and-shut case. Curr Opin Cell Biol 15:266–274.
17. Pickford AS, Cogoni C. 2003. RNA-mediated gene silencing. Cell Mol Life Sci 60:871–882.
18. Chen ES, Saitoh S, Yanagida M, Takahashi K. 2003. A cell cycle-regulated GATA factor promotes centromeric localization of CENP-A in fission yeast. Mol Cell 11:175–187.
19. David G, Turner GM, Yao Y, Protopopov A, DePinho RA. 2003. mSin3-associated protein, mSds3, is essential for pericentric heterochromatin formation and chromosome segregation in mammalian cells. Genes Dev 17:2396–2405.
20. Craig JM, Earle E, Canham P, Wong LH, Anderson M, et al. 2003. Analysis of mammalian proteins involved in chromatin modification reveals new metaphase centromeric proteins and distinct chromosomal distribution patterns. Hum Mol Genet 12:1–13.
21. Sims RJ 3rd, Nishioka K, Reinberg D. 2003. Histone lysine methylation: a signature for chromatin function. Trends Genet 19:629–639.
22. Ahringer J. 2000. NuRD and SIN3 histone deacetylase complexes in development. Trends Genet 16:351–356.
23. Kouzarides T. 2002. Histone methylation in transcriptional control. Curr Opin Genet Dev 12:198–209.
24. Ayer DE. 1999. Histone deacetylases: transcriptional repression with SINers and NuRDs. Trends Cell Biol 9:193–198.
25. Narlikar GJ, Fan HY, Kingston RE. 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell 108:475–487.
26. Jenuwein T, Allis CD. 2001. Translating the histone code. Science 293:1074–1080.
27. Cao R, Zhang Y. 2004. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 14:155–164.
28. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, et al. 2001. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 107:323–337.
29. Fournier C, Goto Y, Ballestar E, Delaval K, Hever AM, et al. 2002. Allele-specific histone lysine methylation marks regulatory regions at imprinted mouse genes. EMBO J 21:6560–6570.
30. Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, et al. 2003. Role of histone H3 lysine 27 methylation in X inactivation. Science 300:131–135.
31. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, et al. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298:1039–1043.
32. Kourmouli N, Jeppesen P, Mahadevhaiah S, Burgoyne P, Wu R, et al. 2004. Heterochromatin and tri-methylated lysine 20 of histone H4 in animals. J Cell Sci 117:2491–2501.
33. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, et al. 2004. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev 18:1251–1262.
34. Kellum R. 2003. HP1 complexes and heterochromatin assembly. Curr Top Microbiol Immunol 274:53–77.
35. Li Y, Kirschmann DA, Wallrath LL. 2002. Does heterochromatin protein 1 always follow code? Proc Natl Acad Sci USA 99 Suppl 4:16462–16469.
36. Saveliev A, Everett C, Sharpe T, Webster Z, Festenstein R. 2003. DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing. Nature 422:909–913.
37. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410:116–120.
38. Taddei A, Maison C, Roche D, Almouzni G. 2001. Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. Nat Cell Biol 3:114–120.
39. Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, Nakatani Y. 2002. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. Science 296:1132–1136.
40. Bailis JM, Forsburg SL. 2003. It's all in the timing: linking S phase to chromatin structure and chromosome dynamics. Cell Cycle 2:303–306.
41. Hayakawa T, Haraguchi T, Masumoto H, Hiraoka Y. 2003. Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase. J Cell Sci 116:3327–3338.
42. Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, et al. 2003. Maintenance of stable heterochromatin domains by dynamic HP1 binding. Science 299:721–725.
43. Sewalt RG, Gunster MJ, van der Vlag J, Satijn DP, Otte AP. 1999. C-Terminal binding protein is a transcriptional repressor that interacts with a specific class of vertebrate Polycomb proteins. Mol Cell Biol 19:777–787.
44. van der Vlag J, Otte AP. 1999. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nat Genet 23:474–478.
45. Sewalt RG, Lachner M, Vargas M, Hamer KM, den Blaauwen JL, et al. 2002. Selective interactions between vertebrate polycomb homologs and the SUV39H1 histone lysine methyltransferase suggest that histone H3-K9 methylation contributes to chromosomal targeting of Polycomb group proteins. Mol Cell Biol 22:5539–5553.
46. Yamamoto K, Sonoda M, Inokuchi J, Shirasawa S, Sasazuki T. 2004. Polycomb group suppressor of zeste 12 links heterochromatin protein 1alpha and enhancer of zeste 2. J Biol Chem 279:401–406.
47. Wade PA. 2001. Methyl CpG-binding proteins and transcriptional repression. Bioessays 23:1131–1137.
48. Meehan RR. 2003. DNA methylation in animal development. Semin Cell Dev Biol 14:53–65.
49. Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nat Rev Genet 3:662–673.
50. Hutchins AS, Mullen AC, Lee HW, Sykes KJ, High FA, et al. 2002. Gene silencing quantitatively controls the function of a developmental trans-activator. Mol Cell 10:81–91.
51. Fuks F, Hurd PJ, Deplus R, Kouzarides T. 2003. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 31:2305–2312.
52. Selker EU. 1990. DNA methylation and chromatin structure: a view from below. Trends Biochem Sci 15:103–107.
53. Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, et al. 2003. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol 13:1192–1200.
54. Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, et al. 2002. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem 278:4035–4040.
55. Hashimshony T, Zhang J, Keshet I, Bustin M, Cedar H. 2003. The role of DNA methylation in setting up chromatin structure during development. Nat Genet 34:187–192.
56. Ohlsson R, Renkawitz R, Lobanenkov V. 2001. CTCF is a uniquely versatile transcription regulator linked to epigenetics and disease. Trends Genet 17:520–527.
57. Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, et al. 2001. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. Dev Cell 1:749–758.
58. Stirzaker C, Song JZ, Davidson B, Clark SJ. 2004. Transcriptional gene silencing promotes DNA hypermethylation through a sequential change in chromatin modifications in cancer cells. Cancer Res 64:3871–3877.
59. Clark SJ, Melki J. 2002. DNA methylation and gene silencing in cancer: which is the guilty party? Oncogene 21:5380–5387.
60. Fuks F, Burgers WA, Godin N, Kasai M, Kouzarides T. 2001. Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. EMBO J 20:2536–2544.
61. Nielsen AL, Sanchez C, Ichinose H, Cervino M, Lerouge T, et al. 2002. Selective interaction between the chromatin-remodeling factor BRG1

and the heterochromatin-associated protein HP1alpha. EMBO J 21: 5797–5806.
62. Dennis K, Fan T, Geiman T, Yan Q, Muegge K. 2001. Lsh, a member of the SNF2 family, is required for genome-wide methylation. Genes Dev 15:2940–2944.
63. Wade PA, Gegonne A, Jones PL, Ballestar E, Aubry F, et al. 1999. Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat Genet 23:62–66.
64. Chadwick BP, Willard HF. 2002. Cell cycle-dependent localization of macroH2A in chromatin of the inactive X chromosome. J Cell Biol 157:1113–1123.
65. Ladurner AG. 2003. Inactivating chromosomes: a macro domain that minimizes transcription. Mol Cell 12:1–3.
66. Meneghini MD, Wu M, Madhani HD. 2003. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell 112:725–736.
67. Rangasamy D, Berven L, Ridgway P, Tremethick DJ. 2003. Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J 22:1599–1607.
68. Ahmad K, Henikoff S. 2002. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. Mol Cell 9:1191–1200.
69. Grigoryev SA. 2001. Higher-order folding of heterochromatin: protein bridges span the nucleosome arrays. Biochem Cell Biol 79:227–241.
70. Choi CY, Kim YH, Kwon HJ, Kim Y. 1999. The homeodomain protein NK-3 recruits Groucho and a histone deacetylase complex to repress transcription. J Biol Chem 274:33194–33197.
71. Westman BJ, Mackay JP, Gell D. 2002. Ikaros: a key regulator of haematopoiesis. Int J Biochem Cell Biol 34:1304–1307.
72. Lu BY, Emtage PC, Duyf BJ, Hilliker AJ, Eissenberg JC. 2000. Heterochromatin protein 1 is required for the normal expression of two heterochromatin genes in *Drosophila*. Genetics 155:699–708.
73. Gilbert DM. 2002. Replication timing and transcriptional control: beyond cause and effect. Curr Opin Cell Biol 14:377–383.
74. Prioleau MN, Gendron MC, Hyrien O. 2003. Replication of the chicken beta-globin locus: early-firing origins at the 5′ HS4 insulator and the rho- and betaA-globin genes show opposite epigenetic modifications. Mol Cell Biol 23:3536–3549.
75. Hansen RS, Stoger R, Wijmenga C, Stanek AM, Canfield TK, et al. 2000. Escape from gene silencing in ICF syndrome: evidence for advanced replication time as a major determinant. Hum Mol Genet 9:2575–2587.
76. Lo AW, Craig JM, Saffery R, Kalitsis P, Irvine DV, et al. 2001. A 330 kb CENP-A binding domain and altered replication timing at a human neocentromere. EMBO J 20:2087–2096.
77. Bickmore WA, Carothers AD. 1995. Factors affecting the timing and imprinting of replication on a mammalian chromosome. J Cell Sci 108:2801–2809.
78. Craig JM, Wong LH, Lo AWI, Earle E, Choo KHA. 2003. Centromeric chromatin pliability and memory at a human neocentromere. EMBO J 12:3109–3121.
79. McNairn AJ, Gilbert DM. 2003. Epigenomic replication: linking epigenetics to DNA replication. Bioessays 25:647–656.
80. Shibahara K, Stillman B. 1999. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. Cell 96:575–585.
81. Henikoff S. 2003. Versatile assembler. Nature 423:814–815, 817.
82. Reese BE, Bachman KE, Baylin SB, Rountree MR. 2003. The methyl-CpG binding protein MBD1 interacts with the p150 subunit of chromatin assembly factor 1. Mol Cell Biol 23:3226–3236.
83. Milutinovic S, Zhuang Q, Szyf M. 2002. Proliferating cell nuclear antigen associates with histone deacetylase activity, integrating DNA replication and chromatin modification. J Biol Chem 277:20974–20978.
84. Cobbe N, Heck MM. 2000. Review: SMCs in the world of chromosome biology- from prokaryotes to higher eukaryotes. J Struct Biol 129:123–143.
85. Pak DT, Pflumm M, Chesnokov I, Huang DW, Kellum R, et al. 1997. Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. Cell 91:311–323.
86. Huang DW, Fanti L, Pak DT, Botchan MR, Pimpinelli S, et al. 1998. Distinct cytoplasmic and nuclear fractions of Drosophila heterochromatin protein 1: their phosphorylation levels and associations with origin recognition complex proteins. J Cell Biol 142:307–318.
87. Saitoh Y, Miyagi S, Ariga H, Tsutsumi K. 2002. Functional domains involved in the interaction between Orc1 and transcriptional repressor AIF-C that bind to an origin/promoter of the rat aldolase B gene. Nucleic Acids Res 30:5205–5212.
88. Partridge JF, Scott KS, Bannister AJ, Kouzarides T, Allshire RC. 2002. cis-acting DNA from fission yeast centromeres mediates histone H3 methylation and recruitment of silencing factors and cohesin to an ectopic site. Curr Biol 12:1652–1660.
89. Labrador M, Corces VG. 2002. Setting the boundaries of chromatin domains and nuclear organization. Cell 111:151–154.
90. Schlissel M. 2004. The spreading influence of chromatin modification. Nat Genet 36:438–440.
91. Georgel PT, Horowitz-Scherer RA, Adkins N, Woodcock CL, Wade PA, et al. 2003. Chromatin compaction by human MeCP2. Assembly of novel secondary chromatin structures in the absence of DNA methylation. J Biol Chem 278:32181–32188.
92. Thiru A, Nietlispach D, Mott HR, Okuwaki M, Lyon D, et al. 2004. Structural basis of HP1/PXVXL motif peptide interactions and HP1 localisation to heterochromatin. EMBO J 23:489–499.
93. Seum C, Delattre M, Spierer A, Spierer P. 2001. Ectopic HP1 promotes chromosome loops and variegated silencing in *Drosophila*. EMBO J 20:812–818.
94. Istomina NE, Shushanov SS, Springhetti EM, Karpov VL, Krasheninnikov IA, et al. 2003. Insulation of the chicken beta-globin chromosomal domain from a chromatin-condensing protein, MENT. Mol Cell Biol 23:6455–6468.
95. Geiman TM, Sankpal UT, Robertson AK, Chen Y, Mazumdar M, et al. 2004. Isolation and characterization of a novel DNA methyltransferase complex linking DNMT3B with components of the mitotic chromosome condensation machinery. Nucleic Acids Res 32:2716–2729.
96. Isogai Y, Tjian R. 2003. Targeting genes and transcription factors to segregated nuclear compartments. Curr Opin Cell Biol 15:296–303.
97. Weierich C, Brero A, Stein S, von Hase J, Cremer C, et al. 2003. Three-dimensional arrangements of centromeres and telomeres in nuclei of human and murine lymphocytes. Chromosome Res 11:485–502.
98. Matsuda E, Agata Y, Sugai M, Katakai T, Gonda H, et al. 2001. Targeting of Kruppel-associated box-containing zinc finger proteins to centromeric heterochromatin. Implication for the gene silencing mechanisms. J Biol Chem 276:14222–14229.
99. Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG. 1999. Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. Mol Cell 3:207–217.
100. Francastel C, Walters MC, Groudine M, Martin DI. 1999. A functional enhancer suppresses silencing of a transgene and prevents its localization close to centrometric heterochromatin. Cell 99:259–269.
101. Gribnau J, Hochedlinger K, Hata K, Li E, Jaenisch R. 2003. Asynchronous replication timing of imprinted loci is independent of DNA methylation, but consistent with differential subnuclear localization. Genes Dev 17:759–773.
102. Cmarko D, Verschure PJ, Otte AP, van Driel R, Fakan S. 2003. Polycomb group gene silencing proteins are concentrated in the perichromatin compartment of the mammalian nucleus. J Cell Sci 116:335–343.
103. Kunze B, Weichenhan D, Virks P, Traut W, Winking H. 1996. Copy numbers of a clustered long-range repeat determine C-band staining. Cytogenet Cell Genet 73:86–91.
104. Saffery R, Sumer H, Hassan S, Wong LH, Craig JM, et al. 2003. Transcription within a functional human centromere. Mol Cell 12:509–516.
105. Elgin SC, Grewal SI. 2003. Heterochromatin: silence is golden. Curr Biol 13:R895–R898.
106. Maison C, Almouzni G. 2004. HP1 and the dynamics of heterochromatin maintenance. Nat Rev Mol Cell Biol 5:296–304.
107. Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, et al. 2003. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. Mol Cell 12:1577–1589.
108. Ayyanathan K, Lechner MS, Bell P, Maul GG, Schultz DC, et al. 2003. Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. Genes Dev 17:1855–1869.