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Control of gene expression and assembly of chromosomal subdomains by chromatin regulators with antagonistic functions

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Abstract Epigenetic regulation of higher-order chromatin structure controls gene expression and the assembly of chromosomal domains during cell division, differentiation, and development. The proposed “histone code” integrates a complex system of histone modifications and chromosomal proteins that establish and maintain distinctive types of chromatin, such as euchromatin, heterochromatin, and centromeric (CEN) chromatin. The reversible nature of histone acetylation, phosphorylation, and (most recently discovered) methylation are mechanisms for controlling gene expression and partitioning the genome into functional domains. Many different regions of the genome contain similar epigenetic marks (histone modifications), raising the question as to how they are independently specified and regulated. In this review, we will focus on several recent discoveries in chromatin and chromosome biology: (1) identification of long-elusive histone “de-methylating” enzymes that affect chromatin structure, and (2) assembly and maintenance of chromatin domains, specifically heterochromatin and euchromatin, through a dynamic equilibrium of modifying enzymes, histone modifications, and histone variants identified biochemically and genetically.

Introduction

In eukaryotes, linear DNA is packaged into chromatin, a higher-order structure containing histones and nonhistone proteins. Chromatin is generally divided into two cate-

gories: euchromatin and heterochromatin. Euchromatin reflects gene expression, replicates primarily in early to mid S-phase, and has an open conformation that correlates with increased DNase hypersensitivity. Conversely, heterochromatin is highly condensed, contains few genes, replicates late in S-phase, and is stable (i.e., maintained in the same conformation throughout the cell cycle). It has been easy to dismiss heterochromatin as unimportant or “junk DNA,” since it is largely transcriptionally inert. However, it plays important roles in chromosome inheritance, genome stability, and dosage compensation (X inactivation) in mammals. A functional characteristic is its ability to silence gene expression, through a phenomenon originally identified in *Drosophila* and called position effect variegation (PEV) (Karpen 1994). Constitutive heterochromatin is located at highly compacted regions of chromosomes (e.g., centromeres and telomeres), remains condensed throughout the cell cycle, and stably silences gene expression (Allshire et al. 1994; Karpen 1994). However, heterochromatin also exists in a more dynamic state, known as facultative heterochromatin. Facultative heterochromatin is considered “reversible chromatin,” is more variably condensed, and correlates with chromosomal regions that can either be euchromatic or heterochromatic, such as at genes expressed during particular stages of development or differentiation. A classic example is the mammalian inactive X chromosome (Chadwick and Willard 2004), which condenses into the Barr body and shows variable gene expression along the chromosome, often even at the same locus among different females (Carrel and Willard 2005).

Both DNA and chromatin undergo various covalent modifications that collectively contribute to functional marking of the genome. Modifications of the N-terminal tails of histones include acetylation, phosphorylation, ubiquitination, and methylation, and occur at various residues, such as lysine, serine, and arginine. These marks represent epigenetic information, also known as the “histone code,” which enables chromatin to respond to transcriptional or developmental signals, and trigger gene expression and developmental pathways or chromosomal assembly (Jenuwein

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and Allis 2001). Histone modifications can either be repressive or active chromatin markers, but even within these two broad categories, there is a complex regulation of histone combinations that further index chromatin into different functional states (Jenuwein and Allis 2001). Histone acetylation at lysine (K) residues is generally linked with active transcription. In contrast, methylated histones mark both transcriptionally active and silent chromatin, depending on the residue. Methyl groups can be added to both lysines and arginines (R). Lysine can be mono-, di- or trimethylated, while arginine can be mono- or dimethylated (asymmetrically or symmetrically) (Bannister et al. 2002).

Intriguingly, modifications at certain residues inhibit or promote additional modifications at other residues on the same or different histones (Fischle et al. 2003b; Jenuwein and Allis 2001). For instance, H3-K4 methylation inversely corresponds to the methylation of H3-K9 (Litt et al. 2001; Wang et al. 2001a). H3-S10 phosphorylation inhibits H3-K9 methylation, but is linked to K9 and K14 acetylation of H3 (Jenuwein and Allis 2001). Methylation at K4 of H3, phosphorylation at S10 of H3, and H3 acetylation at K9 and K14 are all correlated with gene expression and chromatin with an open conformation (euchromatin) (Jenuwein and Allis 2001). Conversely, H3-K9 methylation and H4-K20 methylation often define condensed chromatin that largely lacks transcriptional activity (heterochromatin) (see below). Multiple levels of chromatin regulation exist within the

modification of a single amino acid residue. H3-K4 can be mono-, di-, or trimethylated. H3-K4 dimethylation is found at regions, such as promoters, that are thought to be “poised” for gene expression, and H3-K4 trimethylation is associated with active transcription (Santos-Rosa et al. 2002; Schneider et al. 2004). Similarly, distinct combinations of methylation states (mono-, di-, or tri-) at the same residue (i.e., H3-K9 and H3-K27) also define different types of chromatin (euchromatin and facultative versus constitutive heterochromatin) (Peters et al. 2003; Rice et al. 2003).

Modification at K4 of H3 initiates a series of events that commit a locus or region to euchromatic assembly. H3-K4 di- and trimethylation by Set1p in *S. cerevisiae* (Table 1) has been shown to trigger recruitment of the chromatin remodeling factor Isw1p, promoting transcription (Santos-Rosa et al. 2003) and preventing the binding of heterochromatic protein Sir3p to euchromatic regions (Santos-Rosa et al. 2004). The addition of methyl groups to H3-K4 works collectively with other histone modifications, such as acetylation, to create an epigenetic signature at a gene or promoter region, marking it for docking by transcription factors. For example, euchromatic genes are both hypermethylated at H3-K4 and H3-K79, and hyperacetylated for H3 and H4 (Schubeler et al. 2004). However, notable exceptions are X-linked and autosomal imprinted genes in which the promoters, but not coding

Table 1 Chromatin regulators discussed in this paper

Modifier	Organism	Activity	Target	Function/location
Htz1	<i>S. cerevisiae</i>	Histone variant	H2A (replacement)	Protects euchromatin; antagonizes Sir protein spreading
H2av	<i>Drosophila</i>	Histone variant	H2A	Pericent het assembly
H2A.Z	Human	Histone variant	H2A	HP1 localization
macroH2A	Human	Histone variant	H2A	X inactivation
Clr4	<i>S. pombe</i>	HMTase	H3-K9	Centromere, mating type locus silencing
<i>Su(var)3-9</i>	<i>Drosophila</i>	HMTase	H3-K9tri	Chromocenter; pericent het
Suv39h1,2	Mouse	HMTase	H3-K9tri	Pericent het
G9A	Human	HMTase	H3-K9di	Pericent het; euchromatin
G9a	Mouse	HMTase	H3-K9di	Pericent het; euchromatin
Set1	<i>S. cerevisiae</i>	HMTase	H3-K4	Euchromatin
SET-7/9	Human	HMTase	H3-K4	Euchromatin; CEN chromatin?
PR-SET7/SET8	Human	HMTase	H4-K20mono	Euchromatin
<i>Su(var)4-20</i>	<i>Drosophila</i>	HMTase	H4-K20tri	Heterochromatin
Suv4-20h1,2	Mouse	HMTase	H4-K20tri	Heterochromatin
<i>E(z)</i>	<i>Drosophila</i>	HMTase	H4-K27	Heterochromatin; euchromatic silencing
EZH2	Human	HMTase	H3-K27	X inactivation
LSD1	Human	Demethylase	H3-K4mo, di	Gene repression
CARM1	Human	HMTase	H3-R17	Gene activation
PAD4/PADI4	Human	Deiminase/citrullinase	Methyl-arg on H3, H4	Gene repression
<i>JIL-1</i> ^{<i>Su(var)3-1</i>}	<i>Drosophila</i>	Acquired <i>Su(var)</i> , histone phosphorylation	H3-S10?	Antagonizes <i>Su(var)3-9</i>

HMTase Histone methyltransferase, *Su(var)* suppressor of variegation, *E(z)* enhancer of zeste, *pericent het* pericentric heterochromatin, *CEN chromatin* centromeric chromatin, *HP1* heterochromatin protein 1, *mo* monomethyl, *di* dimethyl; *tri* trimethyl

regions, of expressed alleles are highly enriched for H3-K4 dimethylation while both the promoters and exons of the silenced alleles are enriched for H3-K9 methylation (Rougeulle et al. 2003). Such distinctions between alleles of the same loci may be important for functionally discriminating or partitioning genes that are biallelically versus monoallelically expressed and establishing or maintaining an epigenetic imprint.

Transcriptional regulation by enzymes that convert or remove histone methyl groups

The opposing activities of acetylases–deacetylases and kinases–phosphatases on histone acetylation and phosphorylation, respectively, monitor gene expression and higher-order structure of euchromatin. Unlike the reversibility of acetylation and phosphorylation of the N-terminal histone tails, methylated histones have been considered highly stable, permanent imprints. Certainly, establishing a stable, heritable chromatin state, through heterochromatin assembly and/or DNA methylation, makes sense to maintain silencing at specific genes or over whole chromosomes (Bannister et al. 2002). However, an outstanding question in the field is how a “stable” modification also plays a more dynamic role in gene activation/repression and chromosome structure. Since gene activity increases when H3-K4 dimethyl becomes H3-K4 trimethyl, the reverse would be a logical mechanism for controlling gene expression by repressing transcription (Santos-Rosa et al. 2002). Historically, several lines of evidence supported the perception that histone methylation leading to heterochromatin formation was a permanent, irreversible mark. First, an active demethylase had yet to be identified. Second, both methylated residues and core histones have similar half-lives. Thus, removal of methyl groups has previously been attributed to either normal histone turnover or histone replacement by unmodified or variant histones during replication. Proteolytic cleavage of N-terminal tails had also been proposed as a mechanism for eliminating methyl marks (Allis et al. 1980; Bannister et al. 2002). However, several new studies have identified enzymes that actively convert methyl groups on arginine and lysine residues of H3 and H4, arguing that histone methylation is also a dynamic state and expanding the potential for epigenetic control of gene expression.

Two groups recently demonstrated that methyl-arginine on histones H3 and H4 is converted to citrulline by peptidylarginine deiminase 4 (PAD4/PADI4) (Cuthbert et al. 2004; Wang et al. 2004). Arginine methylation has been correlated with gene expression and transcriptional activation in response to hormone stimulation. PAD4 is relatively nonspecific and can catalyze conversion of both unmodified and monomethyl-arginines at several sites on the H3 and H4 tails into citrulline. In this reaction, termed “deimination” or “citrullination,” arginine loses an imino group and becomes the amino acid citrulline (Cuthbert et al. 2004; Wang et al. 2004). Furthermore,

citrulline within a histone tail prevents arginine 17 (R17) methylation by CARM1, suggesting that PAD4/PADI4 and/or deimination physically blocks transcriptional activating signals (Cuthbert et al. 2004). The effect of PAD4/PADI4 on gene expression was studied in more detail using the estrogen-regulated pS2 gene. In the absence of estradiol, the pS2 gene is in a transcriptionally active state, and characterized by H3 and H4 arginine methylation within the promoter region (Bauer et al. 2002). Interestingly, PAD4 was present at the promoter; however, the amount of bound PAD4/PADI4 at this site increased several-fold upon hormone induction. The increased occupancy of PAD4/PADI4 at the pS2 promoter was correlated with a decrease in H4-R3 methylation, and an increase in H4-Cit3 (Wang et al. 2004). RNA polymerase II was also uncoupled from the pS2 promoter, suggesting that expression of pS2 was repressed as a result of PAD4/PADI4 binding to prevent the activation signal (Cuthbert et al. 2004). PAD4/PADI4 can also act as a transcriptional repressor when it is directly targeted to a hormone-activated gene (VEGF-A); however, in the case of both genes (pS2 and VEGF-A), it is unclear if PAD4/PADI4 directly affects gene expression *in vivo* or is part of a larger repressor complex that blocks transcription and/or changes chromatin structure.

A caveat of these studies is that they raise several important questions. PAD4/PADI4 cannot deiminate dimethylated arginine. Thus, dimethylated arginine, like trimethylated H3-K4, appears to be a positive signal for transcription. It remains to be determined if dimethyl arginine can be converted to citrulline or is demethylated, and if so, by what mechanism and enzyme. Furthermore, PADI was able to bind to both unmethylated and monomethylated arginines (Cuthbert et al. 2004), shedding some doubt on the functional role of PADI4 in the specific conversion of methyl-arginine. Affinity of PAD4 for unmethylated arginine has not been observed by others, yet its role in pS2 hormone-dependent regulation was demonstrated (Wang et al. 2004). Thus, it can be argued that the activity of PAD/PADI is functionally significant, perhaps primarily in locus-specific regulation of arginine modifications. The function of deiminating/citrullinating enzymes may be multifold, in that the conversion of methyl-arginine to citrulline may itself repress gene activity or PADs/PADIs may completely prevent methylation by histone methyltransferases (HMTases). It is also unclear if they are more broadly implicated in establishing or maintaining silent chromatin domains during development or differentiation (Wang et al. 2004). More detailed studies of citrullinated residues in cellular histones will shed light on the role of demination/citrullination in both gene and chromatin regulation.

The PAD/PADI studies indicated that histone methylation is not the irreversible mark it was thought to be. However, it remained unclear if methyl groups on arginines were the only residues that were dynamically regulated, and if methyl groups could only be altered by conversion rather than complete removal. It was exciting, then, when a new study reported that lysine methylation of H3, a mark of

transcriptionally active chromatin, is specifically reversed by the lysine-specific demethylase 1 (LSD1) (Shi et al. 2004). Human LSD1 is the first histone demethylase to be identified. It is part of multiple chromatin-associated repressor complexes, some of which include histone deacetylases (HDACs). It is also evolutionarily conserved, with putative homologs in *C. elegans*, mouse, *Drosophila*, *Arabidopsis*, and *S. pombe*. LSD1 has homology to FAD-dependent amine oxidases that remove methyl groups through an oxidation reaction that yields an amine and aldehyde (Bannister et al. 2002). Indeed, LSD1 removes methyl groups from dimethylated K4-H3 in vitro and in vivo by FAD-dependent amine oxidation that results in an unmodified histone H3 and formaldehyde (Shi et al. 2004). Depletion of LSD1 by RNAi resulted in increased H3-K4 methylation at promoters and derepression of neuronal genes. Interestingly, LSD1 is specific for only H3-K4 mono- and dimethylation, does not have affinity for trimethylated H3-K4 peptides, and is unable to repress a fully activated promoter. Because H3-K4 dimethylation is a mark of “poised” chromatin (Santos-Rosa et al. 2002), LSD1 may be important for regulating a permissive versus repressive chromatin state before a fully committed functional state marked by H3-K4 trimethylation is established.

In general, trimethylated histones mark fully active (H3-K4 triMe) or fully repressed (H3-K9-triMe) chromatin. These marks may signify inflexible imprints that are stable or irreversible. Alternatively, enzymes that regulate trimethylation may be unique or in different activator or repressor complexes that remained to be identified. It is not clear if LSD1 can remove methyl groups from arginines on H3 and H4, or if there is a demethylase that specifically identifies H3-K4 trimethylation to repress full gene activity. The role of histone demethylation in assembly of silent chromatin domains at centromeres and telomeres, or its role in disease progression, is not known. However, it is clear that histone methylation, such as acetylation and phosphorylation, is a dynamic process that is important for regulating gene expression and chromatin structure.

Regional chromatin regulators: establishment of chromatin domains

Histone demethylase (LSD1, PADs/PADIs) and methylases [*E(z)*/EZH2] may be involved in pathways that target specific promoters or genes (i.e., pS2, VEGF-A) during development or differentiation. These recent studies exemplify how gene expression and chromatin domains may be regulated at local sites. However, historical, as well as new, evidence indicates that chromatin is also regulated as regional subdomains (euchromatin versus heterochromatin) (Ebert et al. 2004). Position effect variegation was first described almost a century ago in *Drosophila* (Muller 1930; Schultz 1936), and has been widely studied in both flies and yeast (Karpen 1994). Muller first showed that treatment of flies with X-rays caused structural rearrangements. Specifically, a rearrangement of the X chromosome placed the white (*w*) gene, which is typically located at the

tip of the X opposite of the centromere, next to centromeric heterochromatin. In flies carrying this inversion, expression of *w* was variably silenced, resulting in a mottled eye phenotype and the hypothesis that heterochromatin was spreading over *w* and silencing its expression (Muller 1930). Since then, dozens of modifiers of position effect variegation [*Mod(var)s*] that suppress [*Su(var)s*] or enhance [*E(var)s*] heterochromatic gene silencing have been identified from genetic screens in *Drosophila* (Table 1). For some of these *Mod(var)s*, the molecular and biochemical functions in chromatin assembly and heterochromatic silencing have been studied. Many of these proteins encode histone modifying enzymes (i.e., histone methyltransferases) or heterochromatic proteins involved in chromatin packaging and gene repression, including *Su(var)3-9*, *Su(var)2-5*, and *Su(var)3-7* (Cleard et al. 1997; Fanti et al. 1998; Schotta et al. 2002, 2004). Ongoing analyses of biochemical activities, molecular structure, cytological localizations, and mutations in these genes have presented an intriguing picture of dynamic chromatin regulation with respect to gene expression or assembly of distinctive chromatin domains.

Heterochromatin: a linear pathway for repressive chromatin assembly

Heterochromatin is epigenetically defined by hypoacetylated histones H3 and H4 and methylated H3-K9, H3-K27, and H4-K20 (Ekwall et al. 1997; Peters et al. 2003; Schotta et al. 2004). In particular, H3-K9 trimethylation, H3-K27 monomethylation, and H4-K20 trimethylation are signatures of constitutive heterochromatin that is typically devoid of gene expression (Peters et al. 2003; Rea et al. 2000; Schotta et al. 2004). Conversely, di- and trimethylation of H3-K9, and trimethylation of H3-K27 characterize facultative heterochromatin that has the potential to be transcriptionally active (Chadwick and Willard 2004; Peters et al. 2002, 2003; Silva et al. 2003). The heterochromatin assembly pathway is initiated by noncoding, double-stranded RNAs (dsRNAs) or short interfering RNAs (siRNAs) and involves the coordinated activities of histone deacetylases and methyltransferases that sequentially recruit heterochromatic proteins, change higher-order chromatin structure, and ultimately silence gene expression (reviewed more extensively in Grewal and Moazed 2003; Grewal and Rice 2004; Lippman and Martienssen 2004).

Many of the chromatin-modifying enzymes involved in heterochromatin assembly were genetically identified as suppressors or enhancers [*Su(var)s* or *E(var)s*] of heterochromatic position effect variegation in *Drosophila*. Some have been shown to be involved biochemically in establishing or maintaining of heterochromatin (Table 1). One such modifier identified in *Drosophila* as a suppressor of heterochromatic PEV is *Su(var)3-9/Suv39h*, a conserved histone methyltransferase that trimethylates K9-H3 (Rea et al. 2000). H3-K9 trimethylation creates a docking site for heterochromatin protein 1 [HP1/*Su(var)2-5* in *Drosophila*],

restricting *Su(var)3-9* to heterochromatic regions and changing chromatin structure (Bannister et al. 2001; Schotta et al. 2002). The structure of HP1, which includes chromo-, hinge, and chromo-shadow domains, suggests that HP1 homodimerizes and acts as a structural bridge that tethers other heterochromatic or silencing proteins and propagates a specialized higher-order structure. At centromeric regions that typically appear cytologically condensed, HP1 has been thought to create a static, highly condensed domain. However, HP1 actually creates a dynamic, yet stable chromatin state, as it transiently associates with and is continually exchanged within heterochromatin (Cheutin et al. 2003). At pericentromeric regions, H3-K9 trimethylation by *Su(var)3-9/Suv39h* and HP1 binding establishes the foundation for additional histone modifications that stabilize heterochromatin, such as H4-K20 trimethylation. The histone methyltransferase activities of PR-Set7/SET8 and *Su(var)4-20/Suv4-20h* have been shown to monomethylate and trimethylate H4-K20, respectively (Fang et al. 2002; Nishioka et al. 2002; Rice et al. 2002; Schotta et al. 2004). Both enzymes were isolated biochemically, rather than through genetic screens for *Su(var)*s. Monomethylated H4-K20 is located throughout chromatin (Nishioka et al. 2002; Rice et al. 2002), whereas H4-K20 trimethylation was found to be enriched in pericentromeric regions in mice and flies (Schotta et al. 2004). H4-K20 trimethyl-

ation is reduced in *Su(var)3-9* mutants, suggesting a dependence of *Su(var)4-20/Suv4-20h* and H4-K20 trimethylation on either H3-K9 trimethylation or *Su(var)3-9* itself (Schotta et al. 2004). Although it has not been directly tested in mammals, HP1 [*Su(var)2-5*] mutants in flies show decreased H4-K20 trimethylation, supporting a sequential pathway for repressive chromatin assembly within the pericentromere (Schotta et al. 2004). Thus, the current model of heterochromatin assembly indicates that once HP1 is stably bound to H3-K9 trimethylated nucleosomes, *Su(var)4-20/Suv4-20h* enzymes are recruited and tethered, and they then trimethylate H4-K20 (Fig. 1). Interestingly, it has been shown that H4-K20 trimethylation and H4-K16 acetylation are mutually exclusive histone modifications, and may represent an important on/off switch for controlling transcriptional activity in euchromatic locations (Nishioka et al. 2002; Sarg et al. 2004).

The paradigm for heterochromatin assembly has been recently extended based on studies focusing on histone replacement and heterochromatin assembly. The H2A histone variant H2Av (H2A.Z in mammals, H2av in *Drosophila*, Htz1 in budding yeast) is enriched in pericentric heterochromatin (Rangasamy et al. 2003; Swaminathan et al. 2005) and interacts with HP1 to compact chromatin (Fan et al. 2004). Evidence supporting its role in initiating heterochromatin assembly came from H2av localization

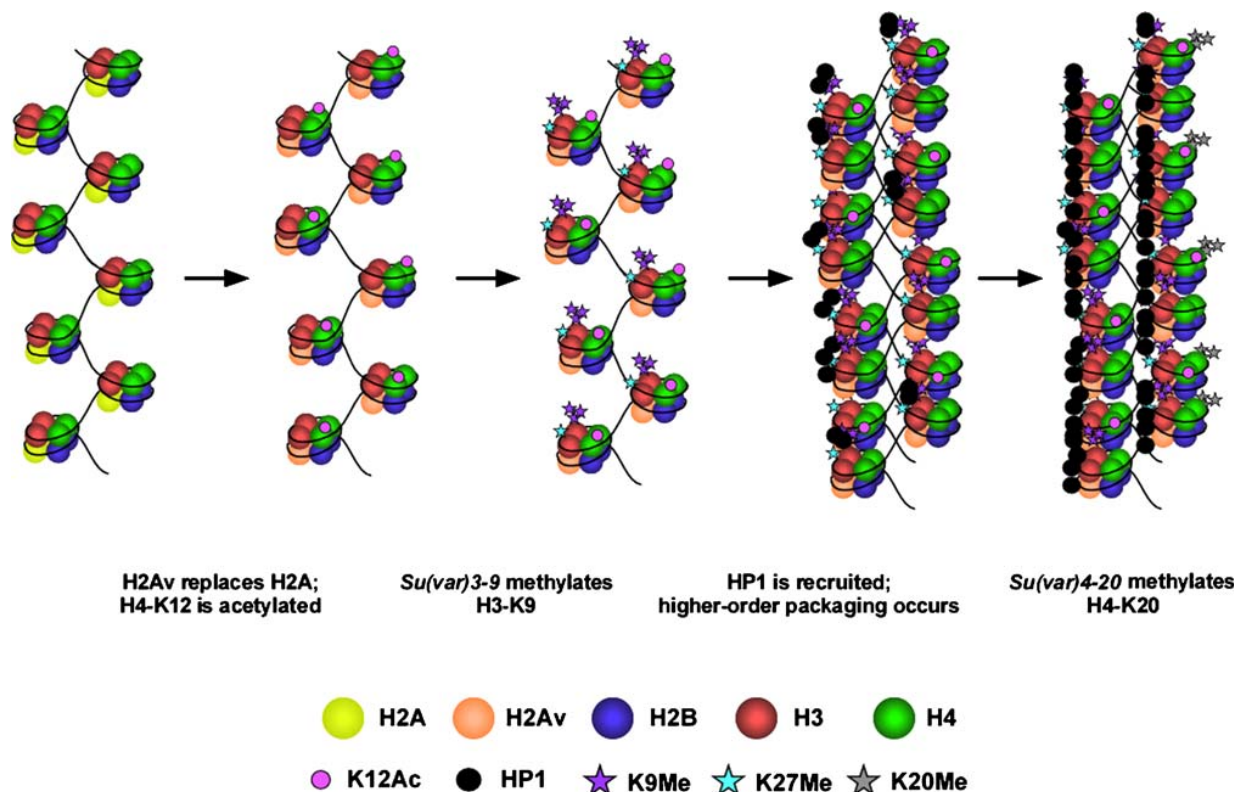


Fig. 1 Proposed pathway for heterochromatin formation at pericentromeric regions. Assembly of constitutive heterochromatin occurs via a linear pathway that is initiated by noncoding RNAs and components of the RNAi pathway (not shown). H2A is exchanged for

the histone variant H2Av, triggering acetylation of H4 at K12. *Su(var)3-9* enzymes methylate K9 of H3 and create binding sites for HP1. HP1 further compacts chromatin and spreads through the region via self-dimerization, and tethers *Su(var)4-20* to trimethylate H4-K20

in *Su(var)3-9* and *Su(var)2-5* *Drosophila* mutants. H2Av localization at the heterochromatic chromocenter on *Drosophila* polytene chromosomes and its effect on heterochromatic silencing is not affected by mutations in *Su(var)3-9* or *Su(var)2-5*. However, *H2Av* mutants were dominant suppressors of PEV and showed reduced H3-K9 methylation and HP1 binding at heterochromatic sites (Swaminathan et al. 2005). H4-K12 acetylation was also reduced at heterochromatin in *H2Av* mutants, but not in *Su(var)2-5* or *Su(var)3-9* mutants, indicating that H2 replacement by H2av and H4-K12 acetylation are early steps in heterochromatin formation, at least in *Drosophila* (Fig. 1). A particularly intriguing finding from this study was that H2Av replacement and its involvement in silencing differs between euchromatin and heterochromatin. H2Av is involved in polycomb-group (PcG)-mediated silencing at euchromatic sites. PcG-associated silencing requires recruitment of *enhancer of zeste* [*E(z)/EZH2*] that methylates H3-K27, and although *H2Av* mutants showed generally reduced levels of PcG protein on chromatin, H3-K27 methylation was not impaired or absent from chromatin. Thus, the dependence of H3 methyl marks on H2A replacement by H2av differs in euchromatin and heterochromatin, and implies that there are different pathways for heterochromatic silencing. In pericentric heterochromatin, histone methylation events are dependent on previous steps in the pathway (i.e., H2Av replacement of H2A; recruitment of HP1 by H3-K9 trimethylation) (Lachner et al. 2001; Schotta et al. 2004), whereas heterochromatic silencing in euchromatic regions may utilize two parallel pathways in which histone methylation occurs independently of H2A replacement by H2Av (Swaminathan et al. 2005).

Antagonistic functions of chromatin regulators

Compelling evidence for antagonistic switching of opposing chromatin states first emerged from studies in *S. cerevisiae*. Sir proteins (Sir2, Sir3, and Sir4) have been shown to be involved in silencing at telomeres and mating-type loci. They interact as a complex with silencers and deacetylated histone tails in nucleosomes to create heterochromatin and to silence gene expression. Spreading of the silencing signal is dependent on Sir2p, which has histone deacetylase activity (Rusche et al. 2003). However, Htz1 (the variant H2A histone in budding yeast) and Sas2, an acetyltransferase that acetylates H4-K16, each oppose the activity of Sir proteins at heterochromatic–euchromatic boundaries in budding yeast. When Sas2 or Htz1 are deleted, Sir proteins spread into euchromatin, causing changes in histone acetylation and H3-K4 methylation that are characteristic of repressive chromatin (Suka et al. 2002; Meneghini et al. 2003). At first glance, these findings in Htz1 mutants appear to contradict the role for H2av/Htz1 in heterochromatin assembly; however, it is thought that Htz1 is a component of “poised” heterochromatin rather than transcriptionally active euchromatin.

These studies have revealed that global changes in histones or histone modifications are important for main-

taining the balance between euchromatin and silenced chromatin. The yeast genome is small, so strict distinctions between expressed and silenced regions may be necessary to ensure genome stability and organism viability. In insects and mammals, centromeres and telomeres are orders-of-magnitude larger than those in yeast, and the boundaries between euchromatin and heterochromatin may be more flexible. In order to understand control of heterochromatin assembly and the roles of different heterochromatic proteins in chromatin structure and gene expression in a larger eukaryote, Ebert et al. (2004) studied multiple alleles of *Su(var)3-9*, the *Drosophila* HP1 homolog. The histone methyltransferase activity and the effect of each allele on gene silencing was evaluated in each mutant using classical PEV rearrangements, such as *In(1)w^{m4}* (red-white mottled eyes). A hypermorphic allele of *Su(var)3-9* was identified that was a strong enhancer of PEV. This mutant also showed increased H3-K9 di- and trimethylation throughout the genome, and, notably, also in many euchromatic regions. Conversely, other mutants were isolated as strong suppressors of PEV, or, in other words, they suppressed gene silencing caused by *Su(var)3-9*. These mutants were alleles of the *Su(var)3-1* gene, which encodes the JIL-1 kinase that in turn controls phosphorylation of H3-S10 within euchromatin (Wang et al. 2001b). The *Su(var)3-1* alleles all contained mutations in the C-terminus of *JIL-1*, and were thus renamed as *JIL-1^{Suvar(3-1)}* mutants. *Su(var)3-1* was the strongest PEV gene to be identified (Ebert et al. 2004) and was shown to suppress gene silencing in seven classical PEV rearrangements (Fig. 2). The most impressive example of the strength of this *Su(var)* was the demonstration that a PEV rearrangement [*T(1;4)w^{m38-21}*] that typically shows complete gene inactivation of *white* and *Notched* in the presence of two extra copies of *Su(var)3-9* was completely derepressed in the presence of *JIL-1^{Suvar(3-1)}*. These striking results show that *JIL-1^{Suvar(3-1)}* completely antagonizes the function of *Su(var)3-9* in heterochromatin assembly and spreading, and acts to maintain a balance between euchromatin and heterochromatin (Fig. 2). This antagonistic relationship appears to support the proposed “binary histone switch hypothesis” (Fischle et al. 2003a,b), in which the functional readout of adjacent histone modifications, such as methylation/phosphorylation (“methyl/phos switch”), influences recruitment of effector or chromatin modifiers. Thus, boundaries between euchromatin and heterochromatin could be established or regulated by the exclusion or inhibition of different histone modifications. For instance, H3-S10 phosphorylation by JIL-1 might act negatively against recruitment of heterochromatin proteins to sites of H3-K9 methylation by *Su(var)3-9*.

Dynamics of other chromatin domains

Facultative chromatin of the inactive X

Collectively, these interesting new studies indicate that chromatin regulation extends beyond indexing the genome into static combinations of histone modifications. Indeed,

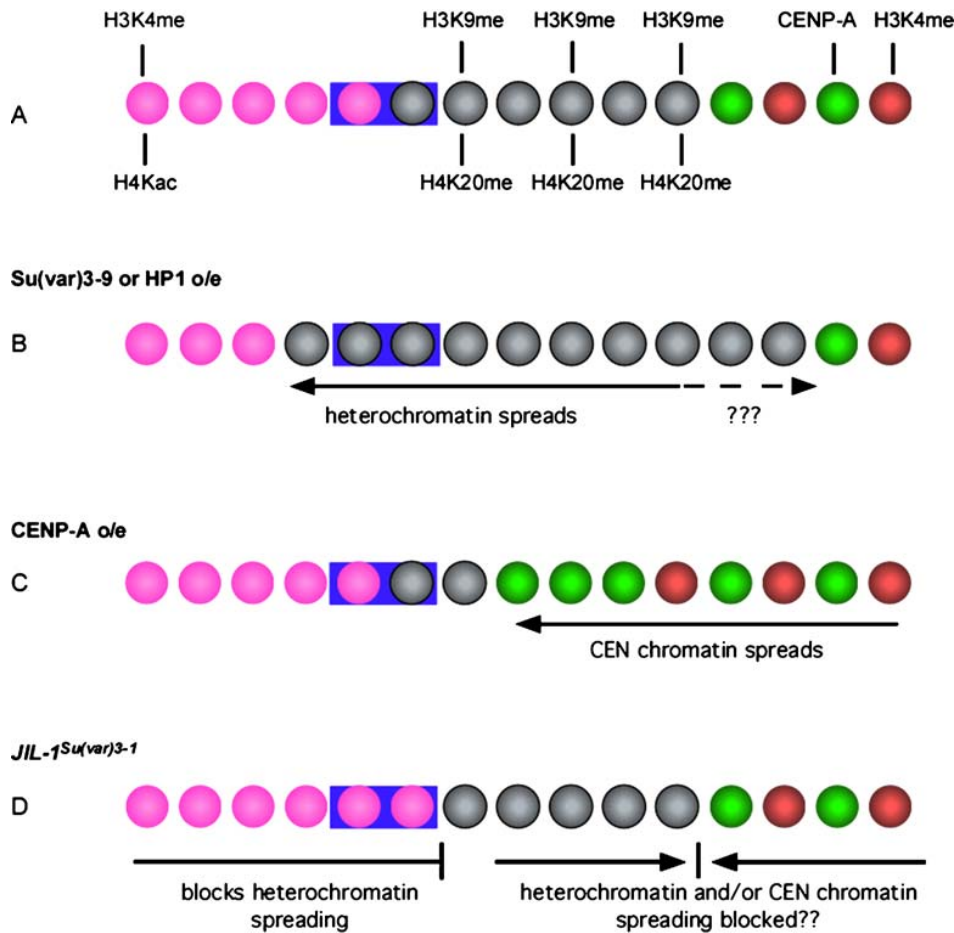


Fig. 2 Dynamic balance between distinct chromatin domains. **a** Schematic representation of a chromatin region near the centromere: euchromatin (pink circles), heterochromatin (gray circles), a position effect variegation allele (PEV) such as *In(1)w^{m4}* (blue rectangle), and centromeric (CEN) chromatin (red and green circles). In *Drosophila*, PEV alleles are variably silenced due to the spread of heterochromatin over the gene (blue rectangle). **b** Overexpression of *Su(var)3-9* or HP1 leads to expansion of heterochromatin into euchromatin. For a *Drosophila* PEV allele (blue rectangle), this results in complete gene silencing. Although it has not been formally demonstrated, we postulate that overexpression of *Su(var)3-9* could also lead to spreading of heterochromatin into the CEN domain (dashed line with

arrow). **c** CENP-A can expand into flanking regions. Overexpression of CENP-A in flies and humans causes CEN chromatin to spread into flanking heterochromatin. In humans, CENP-A can spread up to 500 kb into flanking regions (A. Lam and B. Sullivan, unpublished data). **d** *JIL-1^{Su(var)3-1}* antagonizes *Su(var)3-9*, blocking the expansion of heterochromatin and stabilizing euchromatin. For PEV alleles, this results in complete derepression of silencing so that the gene (blue rectangle) is now fully expressed. We propose that the same, or similar, chromatin modifiers may maintain heterochromatin and CEN chromatin domains by blocking the spread of heterochromatin into CEN chromatin or vice versa

there is an ongoing tightly regulated balance between euchromatin and heterochromatin throughout the genome and during development and differentiation that relies on levels of or communication between proteins that modify histones locally or regionally. Obvious implications from these studies are that other chromosomal regions, such as centromeres and the mammalian inactive X (Xi) chromosome, are also dynamically regulated. Indeed, the Xi consists of interspersed blocks of two chromatin types: one defined by H3-K27 trimethylation (H3-K27 tri-Me) that is catalyzed by EZH2 and the histone variant macroH2A, and the other marked by trimethylated H3-K9 and H4-K20 (Chadwick and Willard 2004; Peters et al. 2002; Silva et al.

2003). One might expect that a dynamic balance in the activities of EZH2, SUV39H/*Suv39h* and *Suv4-20h*, and/or regional boundary elements maintain the distinctions between the subdomains of facultative and constitutive heterochromatin. Intriguingly, gene expression profiles have indicated that, as opposed to be completely active or completely silenced, X-linked genes are variably expressed from different inactive X chromosomes (Carrel and Willard 2005). Thus, a certain level of plasticity must exist in establishing the H3-K27 tri-Me/macroH2A and H3-K9/H4-K20 tri-Me subdomains on Xi (Chadwick and Willard 2004) and may directly correlate to the extent of X-linked gene silencing.

Centromere: a unique chromatin domain within heterochromatin

Centromeric (CEN) chromatin provides the foundation of the kinetochore and is uniquely identified by the centromeric histone H3 variant CENP-A (Sullivan 2001). Intriguingly, CEN chromatin in flies and humans was shown to consist of interspersed nucleosomes of H3 and CENP-A (Blower et al. 2002). The organization of CEN chromatin near heterochromatin appears significant, as this CENP-A: H3 interspersion has since been described in plants and at human neocentromeres (Chueh et al. 2005; Nagaki et al. 2004). Not to be confused with pericentromeric heterochromatin that contains H3-K9 and H4-K20 trimethylation, CEN chromatin has its own distinct chromatin marks. H3 within CEN chromatin is dimethylated at K4, and the entire domain (CENP-A:H3-K4diMe) is flanked by heterochromatin that is marked by H3-K9 di- and trimethylation and H4-K20 trimethylation (Lam and Sullivan, unpublished data; Sullivan and Karpen 2004). Although distinct domains, both CEN chromatin and adjacent heterochromatin that contains HP1 and H3-K9/H4-K20 methylation contribute to chromosome stability (Blower et al. 2002; Peters et al. 2001).

Just as there is a dynamic equilibrium between euchromatin and heterochromatin (Ebert et al. 2004), a similar balance is required between CEN chromatin and flanking heterochromatin. It has been suggested from *Drosophila* studies that heterochromatin serves as a physical boundary, and when it is removed by structural rearrangement, CENP-A spreads into euchromatin (Maggert and Karpen 2001). Furthermore, overexpression of CENP-A in human cells promotes expansion of CEN chromatin into euchromatic sites (Van Hooser et al. 2001). Thus, a balance between CEN chromatin and heterochromatin exists in human cells, even without physically removing or rearranging subdomains. How, then, might the boundary be normally maintained? Perhaps there are secondary and tertiary levels of control, or binary switches, in which euchromatic regulators, such as *JIL-1^{Su(var)3-1}*, *G9a* (the methyltransferase that catalyzes dimethylation of H3-K9 in euchromatin) (Tachibana et al. 2002), or additional chromatin proteins, regulate heterochromatin expansion. In turn, *Su(var)3-9*, *Su(var)4-20* and/or the more stable modification of trimethylation may limit the expansion of CEN chromatin (Fig. 2). Alternatively, because H3 within CEN chromatin has some features of euchromatin, euchromatic regulators or unique modifiers of CEN chromatin may restrict spreading of heterochromatin into CEN chromatin. It will be interesting to determine how the structural architecture and histone modifying proteins participate in the balance between chromatin domains in and around the centromeric chromatin domain.

The identification new chromatin modifying enzymes and antagonistic regulators of chromatin states have elevated chromatin biology and the histone code to a new level

of complexity. Future studies will be important for integrating these new findings into the genetic and molecular hierarchy that controls gene expression and the local and regional assembly and maintenance of chromatin subdomains.

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References

- Allis CD, Bowen JK, Abraham GN, Glover CV, Gorovsky MA (1980) Proteolytic processing of histone H3 in chromatin: a physiologically regulated event in tetrahymena micronuclei. *Cell* 20:55–64
- Allshire RC, Javerzat JP, Redhead NJ, Cranston G (1994) Position effect variegation at fission yeast centromeres. *Cell* 76:157–169
- Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, Kouzarides T (2001) Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* 410:120–124
- Bannister AJ, Schneider R, Kouzarides T (2002) Histone methylation: dynamic or static? *Cell* 109:801–806
- Bauer UM, Daujat S, Nielsen SJ, Nightingale K, Kouzarides T (2002) Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO Rep* 3:39–44
- Blower MD, Sullivan BA, Karpen GH (2002) Conserved organization of centromeric chromatin in flies and humans. *Dev Cell* 2:319–330
- Carrel L, Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature* 434:400–404
- Chadwick BP, Willard HF (2004) Multiple spatially distinct types of facultative heterochromatin on the human inactive X chromosome. *Proc Natl Acad Sci U S A* 101:17450–17455
- Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 299:721–725
- Chueh AC, Wong LH, Wong N, Choo KH (2005) Variable and hierarchical size distribution of L1-retroelement-enriched CE NP-A clusters within a functional human neocentromere. *Hum Mol Genet* 14:85–93
- Cleard F, Delattre M, Spierer P (1997) SU(VAR)3-7, a *Drosophila* heterochromatin-associated protein and companion of HP1 in the genomic silencing of position-effect variegation. *EMBO J* 16:5280–5288
- Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ, Kouzarides T (2004) Histone deimination antagonizes arginine methylation. *Cell* 118:545–553
- Ebert A, Schotta G, Lein S, Kubicek S, Krauss V, Jenuwein T, Reuter G (2004) Su(var) genes regulate the balance between euchromatin and heterochromatin in *Drosophila*. *Genes Dev* 18:2973–2983
- Ekwall K, Olsson T, Turner BM, Cranston G, Allshire RC (1997) Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. *Cell* 91:1021–1032
- Fan JY, Rangasamy D, Luger K, Tremethick DJ (2004) H2A.Z alters the nucleosome surface to promote HP1 alpha-mediated chromatin fiber folding. *Mol Cell* 16:655–661

- Fang J, Feng Q, Ketel CS, Wang H, Cao R, Xia L, Erdjument-Bromage H, Tempst P, Simon JA, Zhang Y (2002) Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. *Curr Biol* 12:1086–1099
- Fanti L, Giovinazzo G, Berloco M, Pimpinelli S (1998) The heterochromatin protein 1 prevents telomere fusions in *Drosophila*. *Mol Cell* 2:527–538
- Fischle W, Wang Y, Allis CD (2003a) Binary switches and modification cassettes in histone biology and beyond. *Nature* 425:475–479
- Fischle W, Wang Y, Allis CD (2003b) Histone and chromatin crosstalk. *Curr Opin Cell Biol* 15:172–183
- Grewal SI, Moazed D (2003) Heterochromatin and epigenetic control of gene expression. *Science* 301:798–802
- Grewal SI, Rice JC (2004) Regulation of heterochromatin by histone methylation and small RNAs. *Curr Opin Cell Biol* 16:230–238
- Jenuwein T, Allis CD (2001) Translating the histone code. *Science* 293:1074–1080
- Karpen GH (1994) Position-effect variegation and the new biology of heterochromatin. *Curr Opin Genet Dev* 4:281–291
- 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
- Lippman Z, Martienssen R (2004) The role of RNA interference in heterochromatic silencing. *Nature* 431:364–370
- Litt MD, Simpson M, Gaszner M, Allis CD, Felsenfeld G (2001) Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. *Science* 293:2453–2455
- Maggert KA, Karpen GH (2001) The activation of a neocentromere in *Drosophila* requires proximity to an endogenous centromere. *Genetics* 158:1615–1628
- 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
- Muller HJ (1930) Types of visible variations induced by X-rays in *Drosophila*. *J Genet* 22:299–335
- Nagaki K, Cheng Z, Ouyang S, Talbert PB, Kim M, Jones KM, Henikoff S, Buell CR, Jiang J (2004) Sequencing of a rice centromere uncovers active genes. *Nat Genet* 36:138–145
- Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang Y, Chuikov S, Valenzuela P, Tempst P, Steward R et al (2002) PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol Cell* 9:1201–1213
- Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, Weipoltshammer K, Pagani M, Lachner M, Kohlmaier A et al (2001) Loss of the *su(Hw)* histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* 107:323–337
- Peters AH, Mermoud JE, O'Carroll D, Pagani M, Schweizer D, Brockdorff N, Jenuwein T (2002) Histone H3 lysine 9 methylation is an epigenetic imprint of facultative heterochromatin. *Nat Genet* 30:77–80
- Peters AH, Kubicek S, Mechtler K, O'Sullivan RJ, Derijck AA, Perez-Burgos L, Kohlmaier A, Opravil S, Tachibana M, Shinkai Y et al (2003) Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol Cell* 12:1577–1589
- 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
- Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 406:593–599
- Rice JC, Nishioka K, Sarma K, Steward R, Reinberg D, Allis CD (2002) Mitotic-specific methylation of histone H4 Lys 20 follows increased PR-Set7 expression and its localization to mitotic chromosomes. *Genes Dev* 16:2225–2230
- Rice JC, Briggs SD, Ueberheide B, Barber CM, Shabanowitz J, Hunt DF, Shinkai Y, Allis CD (2003) Histone methyltransferases direct different degrees of methylation to define distinct chromatin domains. *Mol Cell* 12:1591–1598
- Rougeulle C, Navarro P, Avner P (2003) Promoter-restricted H3 Lys 4 di-methylation is an epigenetic mark for monoallelic expression. *Hum Mol Genet* 12:3343–3348
- Rusche LN, Kirchmaier AL, Rine J (2003) The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. *Annu Rev Biochem* 72:481–516
- Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, Kouzarides T (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* 419:407–411
- Santos-Rosa H, Schneider R, Bernstein BE, Karabetsou N, Morillon A, Weise C, Schreiber SL, Mellor J, Kouzarides T (2003) Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. *Mol Cell* 12:1325–1332
- Santos-Rosa H, Bannister AJ, Dehe PM, Geli V, Kouzarides T (2004) Methylation of H3 lysine 4 at euchromatin promotes Sir3p association with heterochromatin. *J Biol Chem* 279:47506–47512
- Sarg B, Helliger W, Talasz H, Koutzamani E, Lindner HH (2004) Histone H4 hyperacetylation precludes histone H4 lysine 20 trimethylation. *J Biol Chem* 279:53458–53464
- Schneider R, Bannister AJ, Myers FA, Thorne AW, Crane-Robinson C, Kouzarides T (2004) Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. *Nat Cell Biol* 6:73–77
- Schotta G, Ebert A, Krauss V, Fischer A, Hoffmann J, Rea S, Jenuwein T, Dorn R, Reuter G (2002) Central role of *Drosophila* SU(VAR)3-9 in histone H3-K9 methylation and heterochromatic gene silencing. *EMBO J* 21:1121–1131
- Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, Reinberg D, Jenuwein T (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* 18:1251–1262
- Schubeler D, MacAlpine DM, Scalzo D, Wirbelauer C, Kooperberg C, van Leeuwen F, Gottschling DE, O'Neill LP, Turner BM, Delrow J et al (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev* 18:1263–1271
- Schultz J (1936) Variegation in *Drosophila* and the inert chromosomal regions. *Proc Natl Acad Sci U S A* 22:27–33
- Shi Y, Lan F, Matson C, Mulligan P, Whetstone JR, Cole PA, Casero RA (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119:941–953
- Silva J, Mak W, Zvetkova I, Appanah R, Nesterova TB, Webster Z, Peters AH, Jenuwein T, Otte AP, Brockdorff N (2003) Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed-Enx1 polycomb group complexes. *Dev Cell* 4:481–495
- Suka N, Luo K, Grunstein M (2002) Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. *Nat Genet* 32:378–383
- Sullivan KF (2001) A solid foundation: functional specialization of centromeric chromatin. *Curr Opin Genet Dev* 11:182–188
- Sullivan BA, Karpen GH (2004) Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nat Struct Mol Biol* 11:1076–1083
- Swaminathan J, Baxter EM, Corces VG (2005) The role of histone H2Av variant replacement and histone H4 acetylation in the establishment of *Drosophila* heterochromatin. *Genes Dev* 19:65–76
- Tachibana M, Sugimoto K, Nozaki M, Ueda J, Ohta T, Ohki M, Fukuda M, Takeda N, Niida H, Kato H, Shinkai Y (2002) G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev* 16:1779–1791

- Van Hooser AA, Ouspenski II, Gregson HC, Starr DA, Yen TJ, Goldberg ML, Yokomori K, Earnshaw WC, Sullivan KF, Brinkley BR (2001) Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. *J Cell Sci* 114:3529–3542
- Wang H, Cao R, Xia L, Erdjument-Bromage H, Borchers C, Tempst P, Zhang Y (2001a) Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. *Mol Cell* 8:1207–1217
- Wang Y, Zhang W, Jin Y, Johansen J, Johansen KM (2001b) The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. *Cell* 105:433–443
- Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y et al (2004) Human PAD4 regulates histone arginine methylation levels via demethyliminium. *Science* 306:279–283